

國立臺灣海洋大學食品科學系碩士班  
專題討論書面報告

藻類蛋白水解物中血管升壓素轉換酶 (ACE) 抑制胜肽之  
分子對接分析及其抗高血壓的作用

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# Angiotensin I-Converting Enzyme (ACE) Inhibitory Peptides of Molecular Docking Analysis and Antihypertensive Effect of Algae Protein Hydrolysates

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## Abstract

ACE, a component of the renin-angiotensin system, is a zinc metalloprotease that catalyzes cleavage of the C-terminal dipeptide from Ang I to produce the potent vasopressor octapeptide Ang II. Seaweed is a sustainable protein source in the production of peptide-based drugs and functional foods to prevent disease, especially cardiovascular diseases and diabetes. *Pyropia pseudolinearis* protein hydrolysates were purified by RP-HPLC and MALDI-TOF/MS/MS. The major peptide sequence LRM exhibited the highest ACE inhibitory activity ( $IC_{50} = 0.15 \mu M$ ). Docking simulation analyses showed that three peptides were coordinated in the active site of the ACE. However, these peptides do not bind to the zinc ion, which may be the reason for their relatively low ACE inhibitory activity. *Spirulina platensis* protein hydrolysates (SPH) were purified by SEC-HPLC and HPLC-MS. SPH were obtained for VTY ( $IC_{50} = 23.39 \mu M$ ) and LGVP ( $IC_{50} = 45.76 \mu M$ ) were with relatively low  $IC_{50}$  values against ACE. At the ACE active site in the presence of Zn (II), with binding energies of -6.48 kcal/mol and -5.57 kcal/mol, respectively. The results of the docking simulation investigation suggested that VTY displayed a strong binding power to the active pocket S1 (Ala354, Glu384, and Tyr523) and S2 (Gln281, His513, and Tyr520) of ACE via hydrogen bonds, and LGVP only interacted with the S1 pocket (Ala354 and Tyr523). *Gracilaria tenuistipitata* microalgae was successfully purified and identify a novel peptide, MQDAITSVINAADVQGK exhibited significant inhibitory activity on ACE and an inhibition rate of 95.4%. *In vivo* data showed that peptide administration of significantly reduced systolic blood pressure (SBP) in SHRs. In conclusion, the purified peptides isolated from algae protein hydrolysates have potential antihypertensive effect which could potentially be used as functional food ingredients.

## 1 一、前言

2 心血管疾病每年導致超過 1800 萬人死亡，約佔全球死亡人數的三分之一  
3 (Han *et al.*, 2019)。腎素-血管收縮素系統 (Renin-angiotensin system, RAS) 為高血壓形成  
4 原因之一，血管收縮素 I 轉換酶 (Angiotensin I converting enzyme, ACE) 是一種鋅金屬  
5 蛋白酶，可將十肽血管收縮素 I (Angiotensin I) 催化成八肽血管收縮素 II (Angiotensin  
6 II) 與降解緩激肽 (Bradykinin)，從而導致血管收縮，因此抑制體內 ACE 活性不僅具有  
7 調節血壓的效果，亦可降低罹患心血管疾病之風險 (Vickers *et al.*, 2002; Miralles *et al.*,  
8 2018)。目前常見的 ACE 抑制藥物如 Captopril、Enalapril 及 Lisinopril，但這些藥物會  
9 伴隨著不良副作用，如乾咳 (Hu *et al.*, 2023)、皮疹 (Wolf *et al.*, 1990)、血管性水腫 (Kostis  
10 *et al.*, 2018) 等。

11 海藻是生產胜肽藥物和功能性食品的可持續蛋白質來源，可預防心血管疾病與糖尿  
12 病 (Daud *et al.*, 2016)。先前研究指出，藻類已被做為萃取 ACE 抑制能力之活性胜肽的  
13 原料，如石蓴 (*Ulva prolifera*) ( $IC_{50} = 10.32 \mu M$ ) (Li *et al.*, 2023) 與馬尾藻 (*Sargassum*  
14 *maclurei*) ( $IC_{50} = 72.24 \mu M$ ) (Zheng *et al.*, 2020)。

15 海藻之蛋白質含量會因品種而有所差異，其中綠藻為 9-26 g/100g，而紅藻為 10-47  
16 g/100g (Fleurence, 1999)。目前常見之萃取方法為透過水與溶劑進行萃取，其具有萃取率  
17 低、溶劑殘留及環境汙染等缺點，而酵素輔助萃取則可提高蛋白質產量，亦可提高原料  
18 的利用率。(Herrero *et al.*, 2006; Bleakley and Hayes, 2017)。

19 分子對接主要是為了研究標靶與配體相互作用的結合模式，可預測抑制劑與酵素結  
20 合位點的親和力和結合構象，以評估氫鍵、疏水相互作用及靜電相互作用等 (Hetényi and  
21 Spoel, 2002)，目前已被應用於開心果水解物 (Li *et al.*, 2014)、牛奶蛋白 (Pan *et al.*, 2011)  
22 及大麻籽蛋白 (Girgih *et al.*, 2014) 中了解抑制胜肽與 ACE 之間的相互作用。

23 綜合上述，本次專題討論將探討條斑紫菜 (*Pyropia pseudolinearis*)、鈍頂螺旋藻  
24 (*Spirulina platensis*, SP) 及細基江蘘 (*Gracilaria tenuistipitata*) 經酵素水解後，測定其  
25  $IC_{50}$  值以判斷其抑制 ACE 之能力，透過純化並鑑定出胜肽序列，再以分子對接分析胜  
26 肽對 ACE 之抑制型態與結合位點，以了解其作用機制。

## 27 二、藻類蛋白水解物中 ACE 抑制胜肽的純化及其作用機制

### 28 2.1 條斑紫菜蛋白水解物之活性胜肽的鑑定及分子對接

29 首先製備條斑紫菜水溶性蛋白 (Water soluble protein, WSP)，並透過十二烷基硫酸  
30 鈉聚丙烯醯胺凝膠電泳 (Sodium dodecyl sulfate polyacrylamide gel electrophoresis, SDS-

1 PAGE) 分析 WSP 之分子量 (Molecular weight, MW) (Fig. 1A)。結果顯示，WSP 的 MW  
2 主要分布於 20 與 55 kDa。接著測定其可吸收光譜 (350-700 nm)，結果顯示 WSP 具  
3 有四個主要波峰分別位於 495、565、615 及 650 nm 下 (Fig. 1B)。其中於 565 nm 下  
4 的吸光值最高，先前研究指出，在大型紅藻江蘺 (*Gracilaria longa*) 中的藻紅蛋白  
5 (Phycocerythrin, PE) 在 565 nm 處檢測到最大吸光值 (D'Agnolo *et al.*, 1994)。因此確定  
6 本研究中 565 nm 下為 PE，顯示 WSP 的主要成分為 PE。

7 接著利用嗜熱菌蛋白酶 (Thermolysin) 將 WSP 於 70°C 下水解 3 hr，獲得條斑  
8 紫菜蛋白水解物，並測定 WSP 與其水解產物的 ACE 抑制活性 (Fig. 1C)。結果顯示，  
9 WSP 的水解物表現出較高的 ACE 抑制能力為 67.7%，顯示水解產物中具有 ACE 抑  
10 制胜肽。

11 利用反相高效液相層析 (Reversed-phase high-performance liquid chromatography, RP-  
12 HPLC) 將 WSP 水解物進行分離 (Fig. 2A)，總共可獲得 36 個波峰，接著分別測量其  
13 ACE 抑制活性 (Fig. 2B)。結果顯示各波峰的活性範圍介於 28-85%。

14 透過基質輔助雷射脫附電離串聯質譜儀 (Matrix-assisted laser desorption ionization-  
15 time of flight-tandem Mass Spectrometer, MALDI-TOF/MS/MS) 分析 36 個波峰的胜肽序  
16 列 (Table 1)。結果顯示共可檢測到 42 種胜肽序列，而 WSP 的主要成分源自藻膽蛋白  
17 (Phycobiliproteins)。因此利用蛋白質體篩選出 7 個來自藻膽蛋白的胜肽序列並測量其  
18 IC<sub>50</sub> 值 (Table 2)。N 端具有芳香族、鹼性及疏水性胺基酸的三胜肽，有更高的 ACE 抑  
19 制能力 (Wu *et al.*, 2006)。依照上述條件從 7 個胜肽序列可篩選出 3 個，分別為 ARY、  
20 YLR 及 LRM，其中以 LRM 表現出最低之 IC<sub>50</sub> 值為 0.15 μM，表示其對 ACE 抑制  
21 能力最高。

22 將上述 3 種胜肽序列進行合成與分子對接模擬試驗 (Fig. 3)。結果顯示 ARY、YLR  
23 及 LRM 與 ACE 之結合能分別為 -155.685、-166.29 及 -140.68 kcal/mol。文獻指出負  
24 結合能越高、能量越低，表示 ACE 與胜肽的複合體穩定性越好 (Biedermann and  
25 Schneider, 2016)。研究指出，ACE 有三個主要的活性位點，分別為 S1 (Ala<sub>354</sub>、Glu<sub>384</sub> 及  
26 Tyr<sub>523</sub>)、S1' (Glu<sub>162</sub>) 及 S2' (Gln<sub>281</sub>、His<sub>253</sub>、Lys<sub>511</sub>、His<sub>513</sub> 及 Tyr<sub>520</sub>)，抑制劑則可以與  
27 其活性位點結合，達到抑制的效果 (Andújar-Sánchez *et al.*, 2004)。Aliskiren 為一種降血  
28 壓藥物，是有效的腎素競爭性抑制劑，對腎素有高親和力，可與 S3 結合位點結合，防  
29 止血管收縮素原 (Angiotensinogen) 被腎素催化成 AngI，而達到降血壓之效果 (Guang  
30 *et al.*, 2012)。分子對接結果顯示，ARY 透過與 S1' 和 S2' 中的 Arg 和 Tyr 結合，LRM

1 則透過與 S1 和 S2 中的 Arg 和 Met 結合，YLR 則是與 S2 和 S3 中的 Leu 和  
2 Arg 結合，然而此三種胜肽均不與  $Zn^{2+}$  作用。與藥物組相比，Lisinopril 可與 S1、S1'  
3 和 S2' 結合；Captopril 則可與 S1' 和 S2' 結合，且能與  $Zn^{2+}$  相互作用。文獻指出，  
4 ACE 抑制劑可以抑制基質金屬蛋白酶 (Matrix metalloproteinases, MMP) 的活性，導致  
5 鋅尿和鋅缺乏 (Cohen and Golik, 2006)。LRM 的結合能最低表示結合的越緊密且與  
6 Lisinopril 一樣可與 S1 活性位點結合，因此與其具有最高抑制活性相呼應。

7 本研究結果顯示，以嗜熱菌蛋白酶水解條斑紫菜並經由純化後可鑑定出 42 種胜肽  
8 序列，其中共有 7 個胜肽序列來自藻膽蛋白，胜肽 LRM 顯示出最低的  $IC_{50}$  值為 0.15  
9  $\mu M$ ，可與 ACE 的 S1 和 S2 活性位點結合，達到降血壓之功效。

## 10 2.2 鈍頂螺旋藻蛋白水解物之活性胜肽的鑑定及分子對接

11 鈍頂螺旋藻 (*Spirulina platensis*, SP) 的主要成分為粗蛋白，含量約佔乾重的 60-  
12 70% (Gupta *et al.*, 2013)，顯示 SP 是萃取活性胜肽的優良蛋白質來源，其主要成份為藻  
13 膽蛋白，由藻藍蛋白 (Phycocyanin, PC) 和別藻藍蛋白 (Allophycocyanin, APC) 組成，  
14 而兩者皆由  $\alpha$  亞基和  $\beta$  亞基組成。首先將藻膽蛋白利用 ProtParam Tool 進行分析  
15 (Table 3)。結果顯示，C-PC- $\alpha$  subunit、C-PC- $\beta$  subunit、APC- $\alpha$  subunit 及 APC- $\beta$  subunit  
16 這四種亞基的必需胺基酸含量高，且其富含疏水性胺基酸 (Ile、Leu、Val、Pro 及 Ala)  
17 和芳香族胺基酸 (Trp 與 Tyr)。根據文獻指出，胜肽的 C 端若具有疏水性胺基酸，特  
18 別是 Val、Leu、芳香族及環狀胺基酸 (Trp、Tyr 及 Pro) 的胜肽具有較高的 ACE 抑制  
19 活性 (Li *et al.*, 2019)。

20 嗜熱菌蛋白酶 (Thermolysin) 會優先水解大體積芳香族胺基酸 (Murray and  
21 FitzGerald, 2007)，釋放出末端具有疏水性或芳香族的低分子量胜肽，有利於與 ACE 結  
22 合，因此本研究利用嗜熱菌蛋白酶水解 SP 蛋白，並以同體積的蒸餾水作為對照組，均  
23 在 70°C 下水解 3 hr。先前研究指出，分子量  $< 3kDa$  的胜肽具有較高的 ACE 抑制活  
24 性 (Lee and Hur, 2017)，因此利用膜過濾將 SP 蛋白水解物與控制組分離出  $< 3kDa$  分  
25 子量的胜肽，分別為 SPH-3 與 SPC-3 並測定其 ACE 抑制率。結果顯示，SPH-3 的抑  
26 制率高於 SPC-3 (34.17%)，為 75.12%。

27 進一步以膠體層析-高效液相層析 (Size-exclusion chromatography- high performance  
28 liquid chromatography, SEC-HPLC) 分離 SPH-3 (Fig. 4A)。結果顯示，在 214 nm 處檢測  
29 到 5 個波峰 (P1-P5)。接著測定每個波峰之 ACE 抑制率 (Fig. 4B)，其中以 P4 之抑制  
30 率最高達 81.85%，因此選擇 P4 進行下一步的胜肽鑑定。

1 利用高效液相層析質譜儀 (High performance liquid chromatography-mass  
2 spectrometry, HPLC-MS) 鑑定出 P4 的組成 (Fig. 5)。結果顯示，共鑑定出 8 種胜肽序  
3 列 (3~6 胜肽)，且大多數胜肽少於 5 個胺基酸。先前研究指出，大多數的 ACE 抑制  
4 胜肽為 2-20 個胺基酸組成，能更容易結合至 ACE 的活性位點上 (Harnedy and  
5 FitzGerald, 2012)。接著使用 Toxin Pred 進行電腦毒性評估 (Table 4)。結果顯示這 8 種  
6 胜肽均被預測為無毒性，其中 VTY 與 LGVP 對 ACE 的抑制效果較高，IC<sub>50</sub> 值為  
7 23.39 與 45.76 μM。

8 因此將 VTY 與 LGVP 進行分子對接以評估其結合模式 (Fig. 6)。結果顯示，其結  
9 合能值分別為 -6.48 與 -5.57 kcal/mol，而負結合能越高，表示 ACE 與胜肽之複合物的  
10 穩定性越好 (Biedermann and Schneider, 2016)；且 VTY 與 LGVP 中的 Valine 與  
11 Leucine 可與 ACE 的 Zn<sup>2+</sup> 形成氫鍵。文獻指出，氫鍵相互作用力在穩定對接複合物與  
12 影響酵素催化反應方面發揮著重要的作用 (Chaudhary et al., 2009; Girgih et al., 2014; Li  
13 et al., 2014)。ACE 主要有三個活性位點，分別為 S1、S2 及 S1' (Natesh et al., 2004)，  
14 VTY 透過氫鍵與 S1 與 S2 結合，LGVP 則僅與 S1 以氫鍵相互作用。

15 本研究結果顯示，利用嗜熱菌蛋白酶水解鈍頂螺旋藻並經由純化後可鑑定出 VTY  
16 與 LGVP 兩種胜肽序列，其 IC<sub>50</sub> 值分別為 23.39 與 45.76 μM，分子對接結果顯示，  
17 VTY 可透過氫鍵與 S1 與 S2 結合，LGVP 則與 S1 相互作用，表現出抑制 ACE 的  
18 效果。

### 19 2.3 細基江蘼蛋白水解物活性胜肽的鑑定及其對 SHR 的抗高血壓作用

20 海藻細胞壁中的多醣透過雙硫鍵交聯，造成萃取蛋白質的困難度較高 (Wijesinghe  
21 and Jeon, 2012)，故本研究將細基江蘼 (*Gracilaria tenuistipitata*) 先以三種醣酶  
22 (Celluclast、Termamyl 及 Viscozyme) 降解細胞壁多醣，再分別以 Alcalase、Neutrased 及  
23 Flavourzyme 於 50°C 下水解 2、4、6、8 及 10 hr，最後再以 Pepsin 與 Trypsin 在 37°C  
24 下水解 2 與 4 hr，而後以 10,000 x g 離心 20 min，最後將水解物凍乾分別得粗江蘼枯  
25 草桿菌蛋白酶水解物 (Crude *Gracilaria tenuistipitata* Alcalase hydrolysate, CGTA)、粗江  
26 蘼中性蛋白酶水解物 (Crude *Gracilaria tenuistipitata* Neutrased hydrolysate, CGTN) 及粗  
27 江蘼風味蛋白酶水解物 (Crude *Gracilaria tenuistipitata* Flavourzyme hydrolysate, CGTF)。

28 首先將水解 2、4、6、8 及 10 hr 的 CGTA、CGTN、CGTF 進行 ACE 抑制能力  
29 測定 (Fig. 7)，結果顯示水解 4 hr 的 CGTN (GTN4H) 具有最高的 ACE 抑制能力為  
30 82.7%。接著將 GTN4H 進行原發性高血壓大鼠 (Spontaneously Hypertensive Rat, SHR)

1 的短期灌食試驗並測量其收縮壓 (Systolic blood pressure, SBP) 的變化，以評估此胜肽之  
2 降血壓能力，本實驗動物為 SHR 大鼠，共有五個組別，分別為對照組 (0 mg/kg BW)、  
3 藥物組 (Quinapril, 5 mg/kg BW)、低劑量組 (L, 10 mg/kg BW)、中劑量組 (M, 50 mg/kg  
4 BW) 及高劑量組 (H, 200 mg/kg BW)，在服藥 24 hr 間隔中每 2 hr 測量一次 SBP (Fig.  
5 8)。結果顯示，SBP 的降低和劑量呈正相關，高劑量組之 SBP 隨服藥時間增加而下降，  
6 但所有組別均在 24 hr 後回復至原始值。而本實驗高劑量組可使 SBP 降低約 25 mmHg，  
7 表示當 GTN4H 的餵食劑量為 200 mg/kg BW 時具有調節血壓之潛力。

8 利用 Sephadex G-25 之膠體過濾層析純化 GTN4H (Fig. 9)。結果顯示，GTN4H 被  
9 分成三個劃分物 (A、B 及 C)，並測定其 ACE 抑制活性 (Table 5)。結果顯示，GTN4H-  
10 A 顯示出最高的 ACE 抑制活性為 69.74%。接著以逆相高效液相層析質譜儀  
11 (Reversed Phase-High Performance Liquid Chromatographic, RP-HPLC) 的 C<sub>18</sub> 半製備型  
12 管柱進行二次純化 (Fig. 10)。從 GTN4H-A 中可分離出五個波峰 (A1-A5)，其中 A4 表  
13 現出最高的 ACE 抑制活性為 95.4%，因此選擇 A4 進行胜肽序列鑑定。

14 以液相層析串聯質譜儀 (Liquid chromatography-tandem mass spectrometry, LC-  
15 MS/MS) 分析 A4 之分子量及胜肽序列 (Fig. 11)。結果顯示，其鑑定出的胜肽序列為  
16 MQDAITSVINAADVQGK，共由 17 個胺基酸組成，分子量為 1776 Da，根據研究指出，  
17 具有 ACE 抑制活性的胜肽大多含 2-20 個胺基酸 (Hamedy and FitzGerald, 2012)，而上  
18 述結果符合此範圍內。

19 本研究結果顯示，GTN4H 的高劑量組 (200 mg/kg BW) 在體內試驗中可使 SHR  
20 大鼠之 SBP 降低約 25 mmHg，而後續將 GTN4H-A4 純化後可鑑定出具有 ACE 抑制  
21 活性的胜肽為 MQDAITSVINAADVQGK，其 ACE 抑制活性為 95.4%，顯示出降血壓  
22 之功效。

### 23 三、結論

24 條斑紫菜鑑定出的胜肽序列為 LRM (IC<sub>50</sub> = 0.15 μM) 可與 ACE 的 S1 和 S2 活  
25 性位點結合，結合能為 -140.68 kcal/mol。鈍頂螺旋藻鑑定出的胜肽序列為 VTY (IC<sub>50</sub> =  
26 23.39 μM) 透過氫鍵與 S1 與 S2 結合，結合能為 -6.48 kcal/mol。細基江蘼在動物實驗  
27 中，高劑量組可使 SBP 降低約 25 mm Hg，表示當 GTN4H 的餵食劑量為 200 mg/kg  
28 BW 時具有調節血壓之潛力，鑑定出的胜肽序列為 MQDAITSVINAADVQGK 其 ACE  
29 抑制活性為 95.4%。由此三篇文獻證實條斑紫菜、鈍頂螺旋藻及細基江蘼經酵素水解後  
30 可萃取降血壓胜肽。

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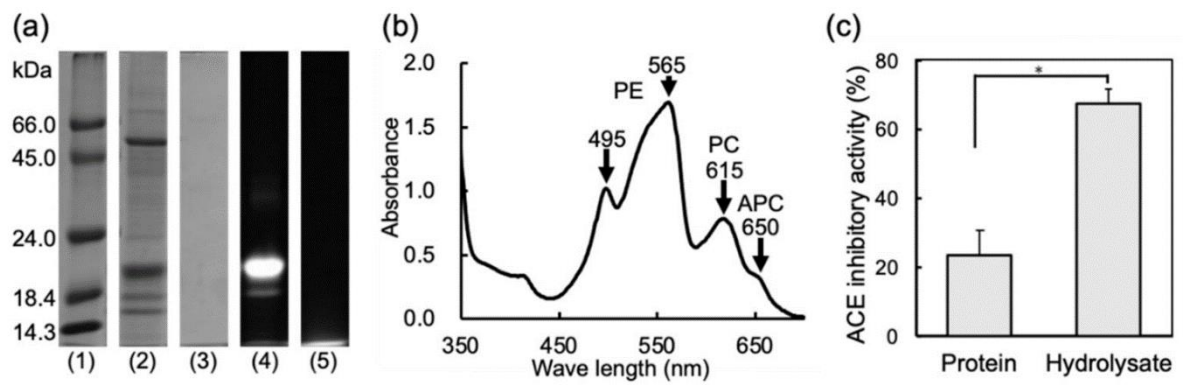
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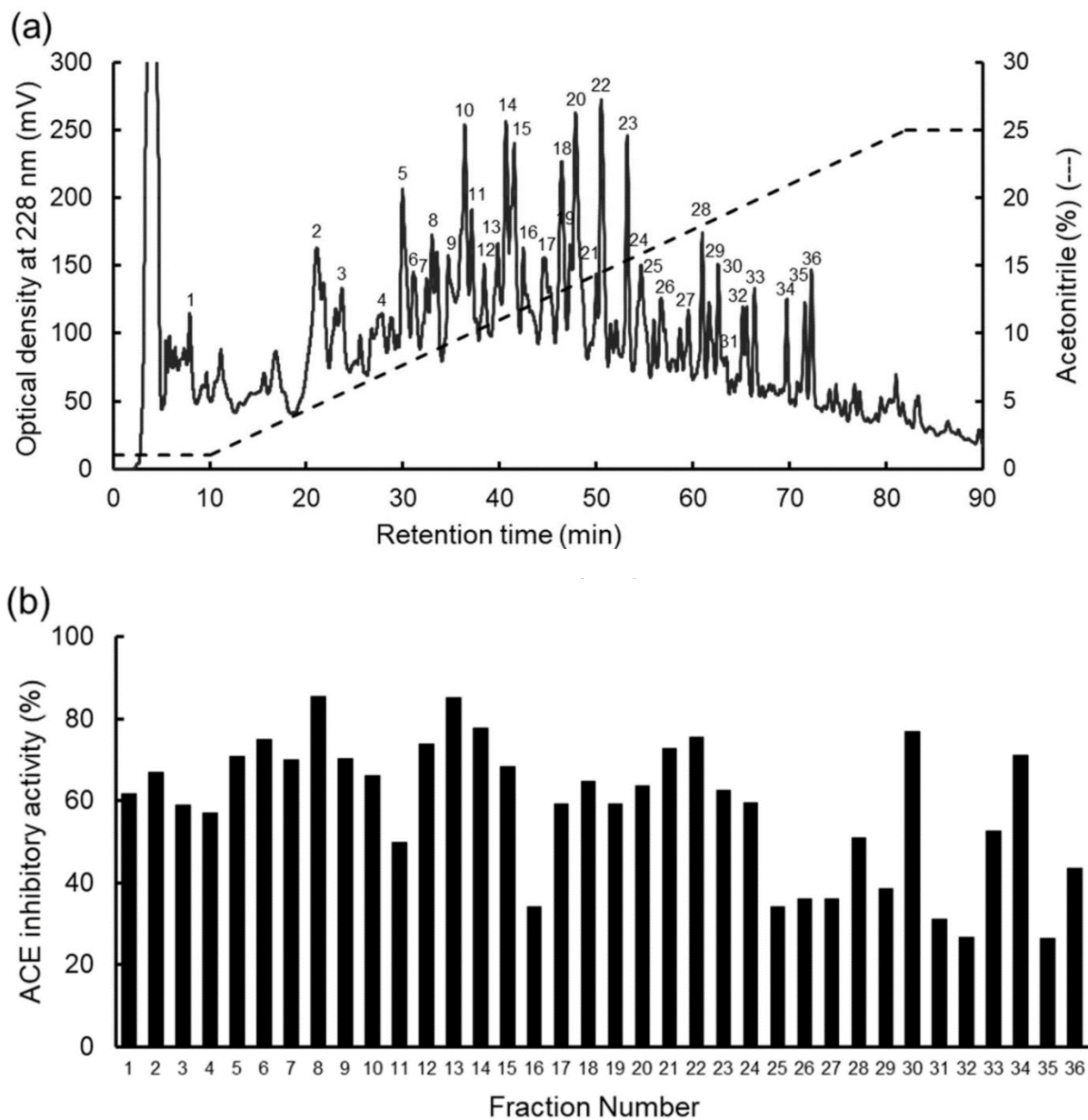
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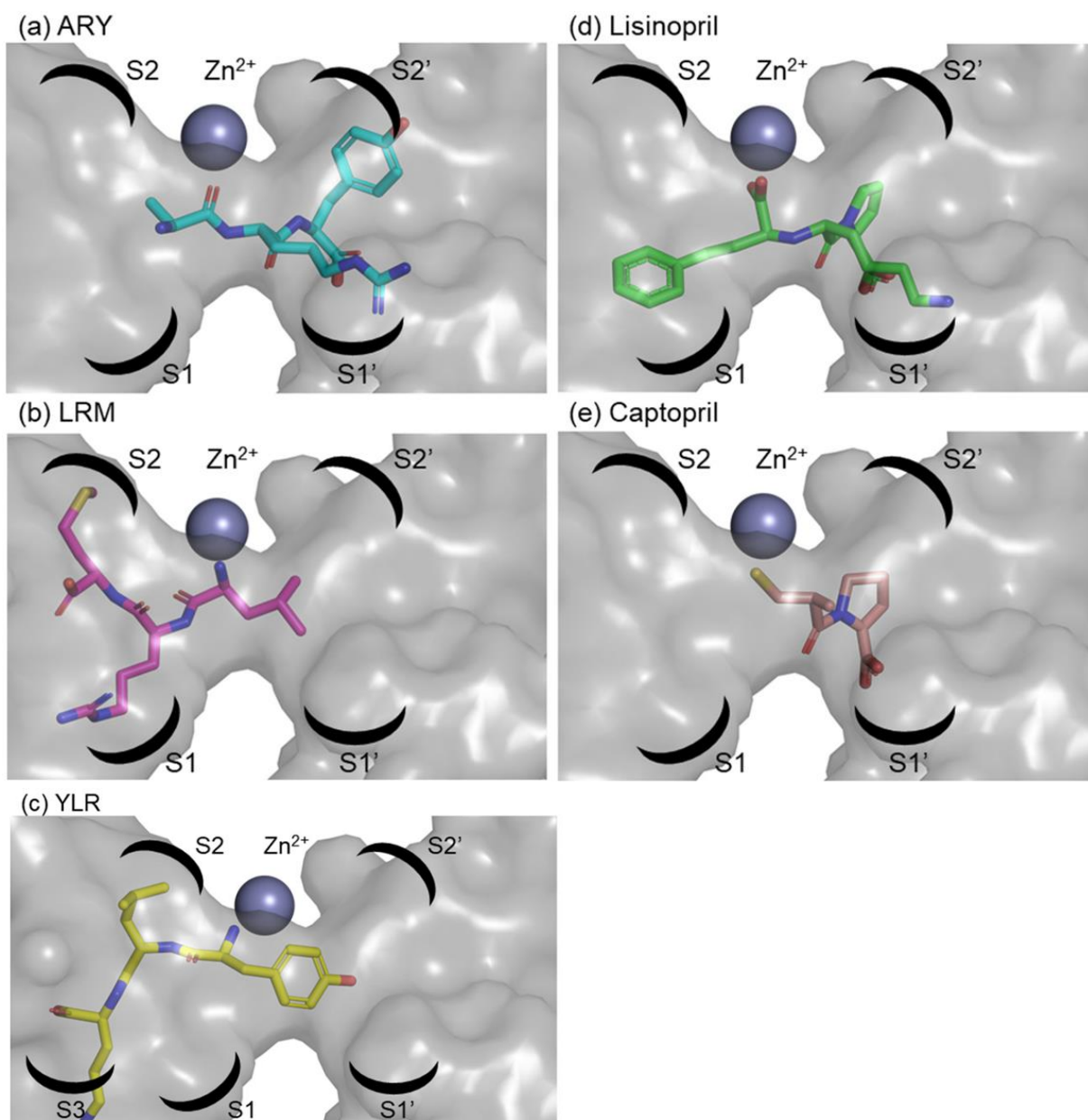
**Fig. 1.** Properties and ACE inhibitory activities of *P. pseudolinearis* WSP and its hydrolysate. (a) SDS-PAGE. Lane 1, Marker; Lane 2, WSP; Lane 3, the thermolysin hydrolysate of the WSP; Lane 4, WSP (Fluorescence); Lane 5, the thermolysin hydrolysate of the WSP (Fluorescence). (b) Visual ray absorption spectra of WSP. (c) ACE inhibitory activities of WSP and the hydrolysate. Bars represent standard errors. \*  $p < 0.05$ .

(Kumagai *et al.*, 2021)



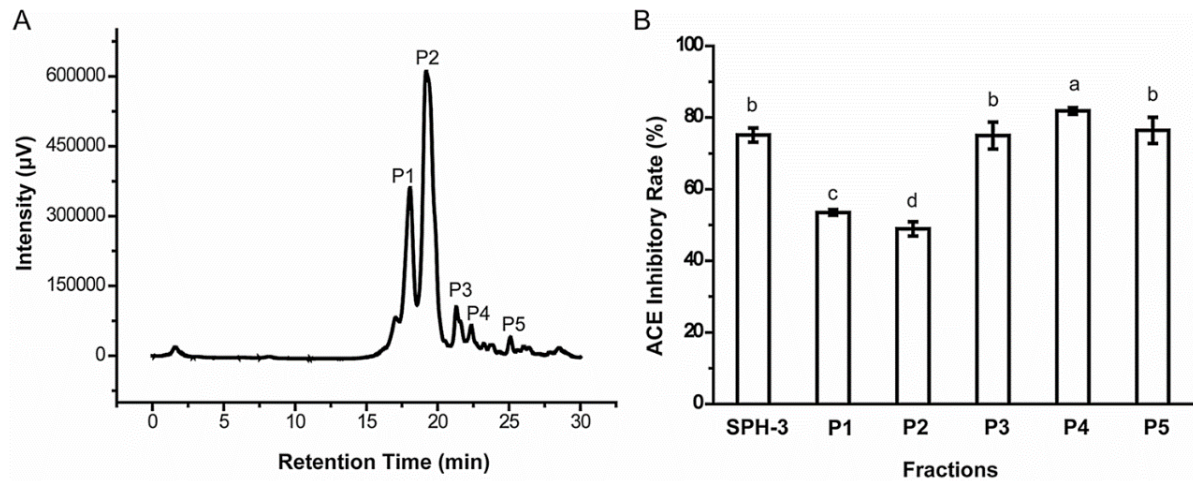
**Fig. 2.** HPLC chromatogram of the WSP hydrolysate and ACE inhibitory activity. (a) Chromatogram of the WSP hydrolysate by HPLC. Peptides were separated by Mightysil RP-18GP column. The numbers on the peaks (1–36) were pooled. (b) ACE inhibitory activity of each fraction (1–36).

(Kumagai *et al.*, 2021)



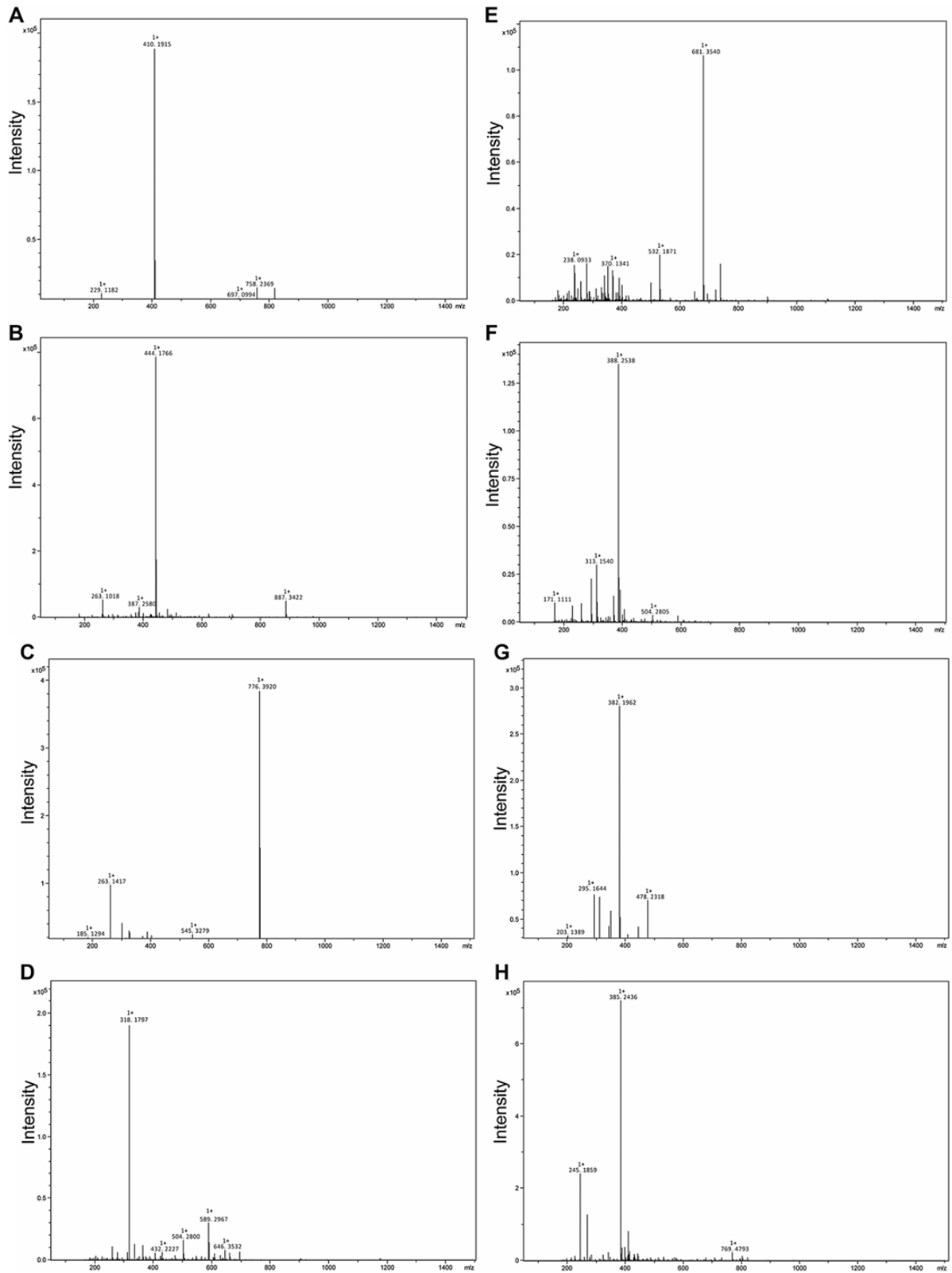
**Fig. 3.** Binding motifs of ARY, LRM, and YLR in the active site of ACE. (a) ARY and ACE interaction; (b) LRM and ACE interaction; (c) YLR and ACE interaction; (d) Lisinopril and ACE interaction (PDB 1O86); (e) Captopril and ACE interaction (PDB 1UZF). Zinc ion is shown as a dark gray sphere. The binding pockets of the enzymes are labeled within the surface-rendered catalytic channel of the C-domain of ACE.

(Kumagai *et al.*, 2021)



**Fig. 4.** Chromatogram of SPH-3 fraction and ACE inhibitory activity of different peak fractions. A Purification from SPH-3 by SEC-HPLC. B ACE inhibitory activity of each peak fraction. Different letters in the same line indicate significant differences ( $p < 0.05$ )

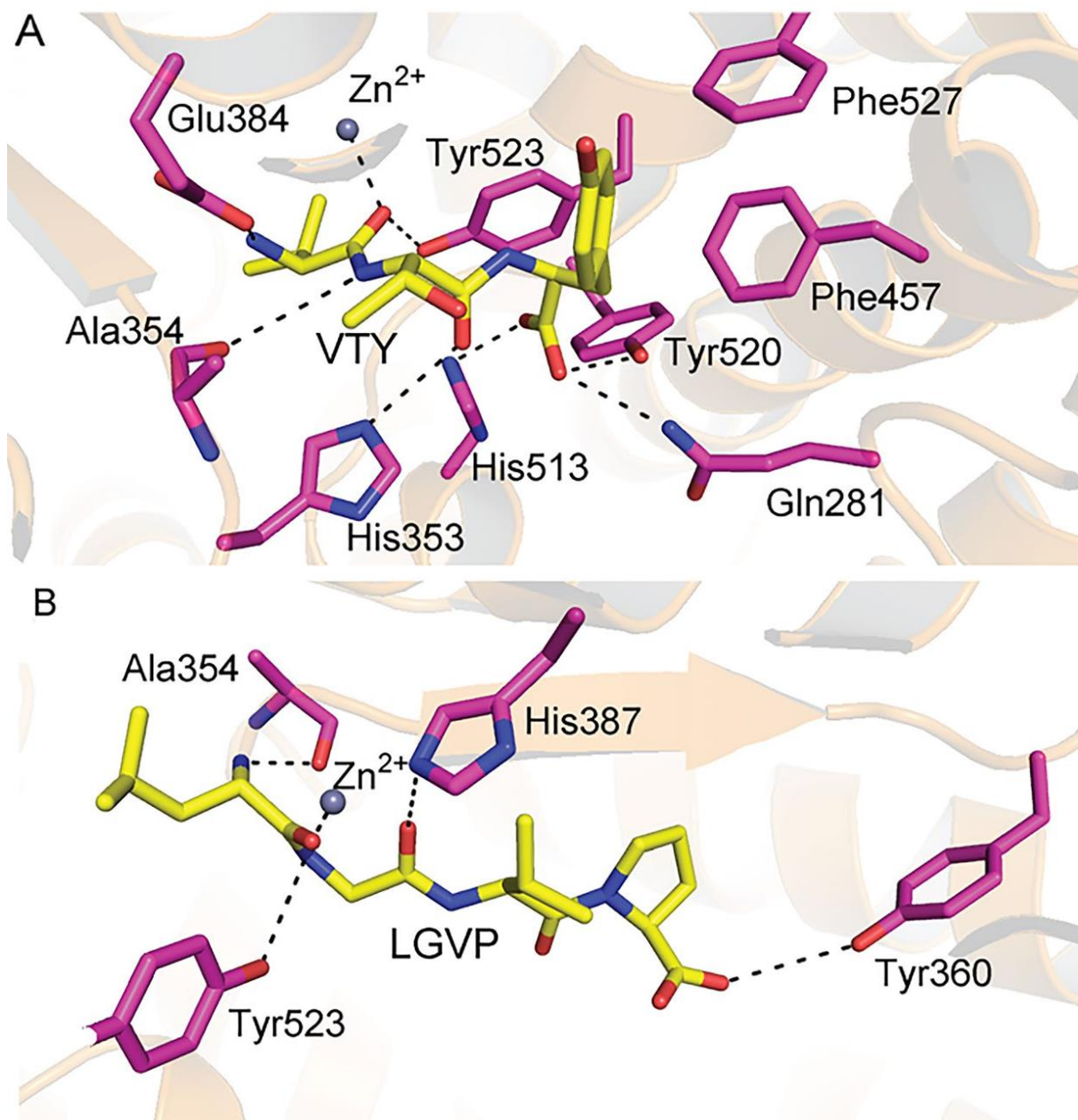
(Zhang *et al.*, 2022)



**Fig. 5.** Mass spectra of peptides identified by HPLC-MS. **A** LDY, **B** FDY, **C** LRDMEI, **D** IDA, **E** LRETY, **F** IQK, **G** VTY, **H** LGVP

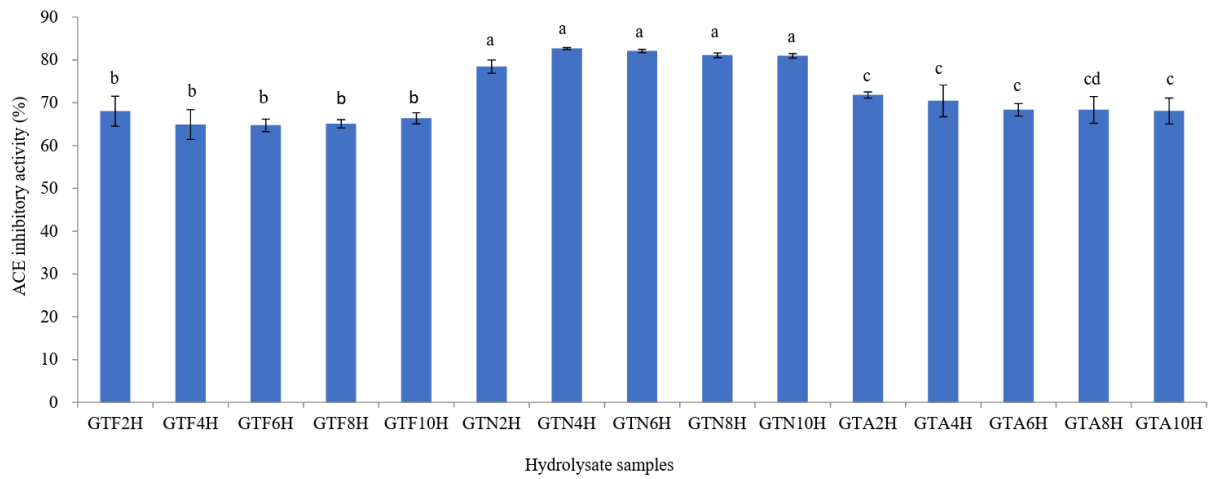
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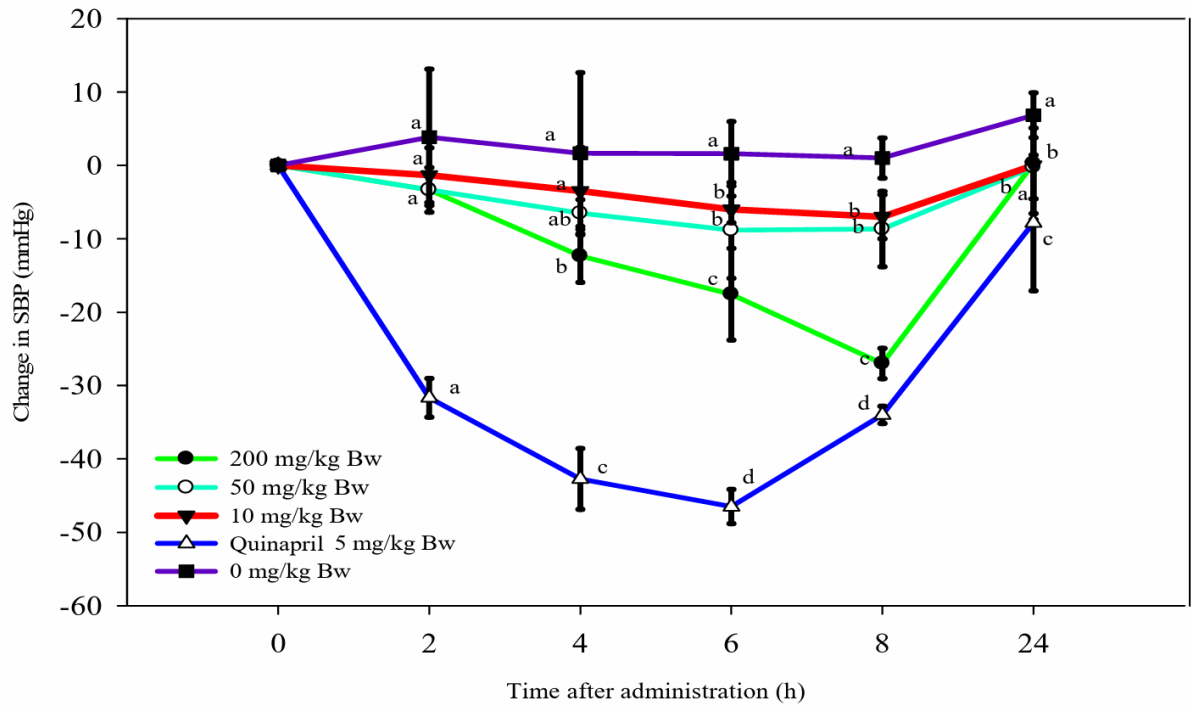
**Fig. 6.** Local overview of the best ranked docking models of VTY (A) and LGVP (B) binding with ACE (PDB: 1O8A) at the ACE catalytic site. The peptides VTY and LGVP are shown as sticks colored in yellow. ACE residues involved in binding peptides are shown as sticks colored in magenta. The possible hydrogen bonds are represented by dashed lines.

(Zhang *et al.*, 2022)



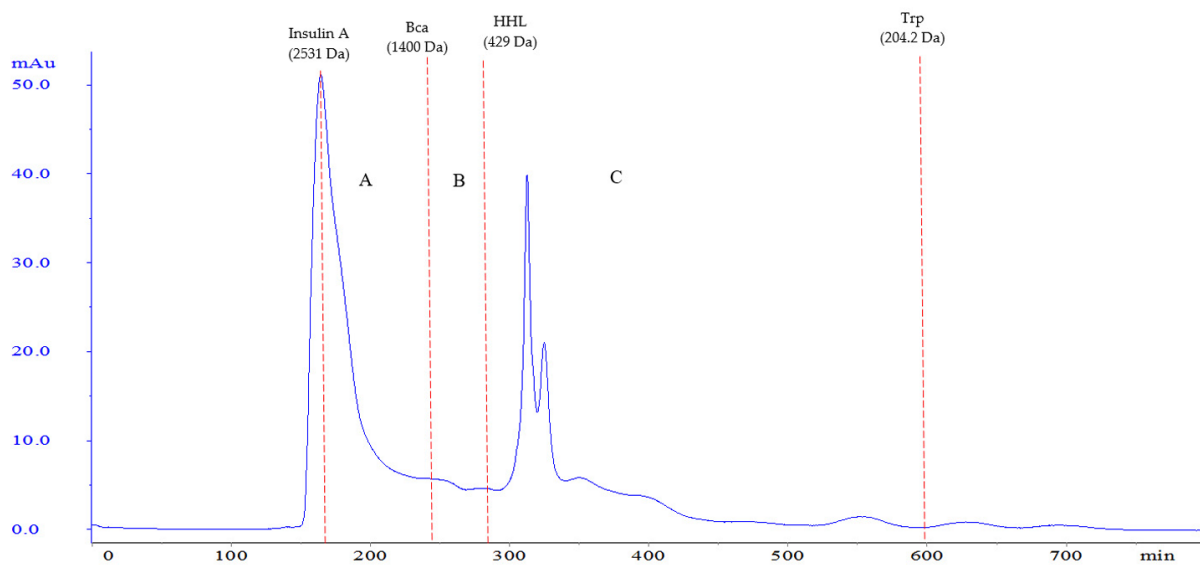
**Fig. 7.** *In vitro* ACE-inhibitory activity of 10 mg/mL of protein hydrolysates from groups GTF, GTN, and GTA. Data represent mean SD (n =3). The different lower-case letters are significant differences ( $p < 0.05$ ).

(Su *et al.*, 2022)



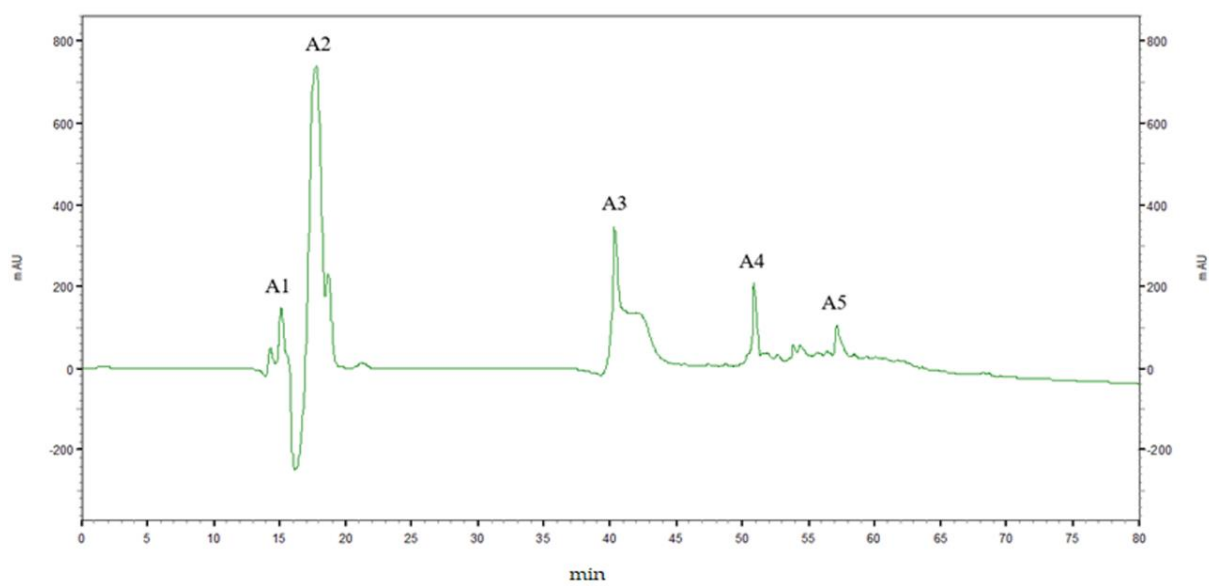
**Fig. 8.** *In vitro* antihypertensive effect of GTN4H and quinapril in SHR. Systolic blood pressure for 24 h after administration (■ 0, ▼ 10, ○ 50, ● 200 mg/kg BW) and compared with Quinapril (△ 5 mg/kgBW). Values are mean SD, n=6. Different lowercase letters within the same time after administration indicate significant differences ( $p < 0.05$ ).

(Su *et al.*, 2022)



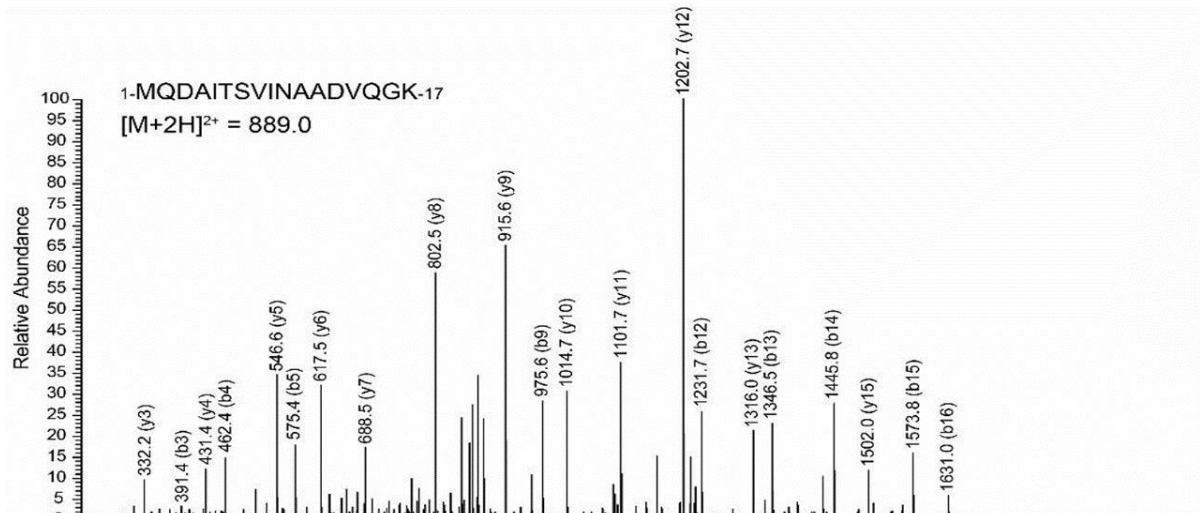
**Fig. 9.** Sephadex G-25 gel filtration chromatography and molecular mass distribution of GTN4H. Insulin A (2531 Da), Bca: bacitracin zinc salt (1400 Da), HHL: hippuryl-histidyl-leucine (429 Da), Trp: tryptophan (204.2 Da). Time: A (160–280 min), B (290–340 min), C (350–440 min).

(Su *et al.*, 2022)



**Fig. 10.** Elution profile of GTN4H-A by C18 reversed-phase HPLC. (Flow rate of 1 mL/min with a 0–80% linear gradient of acetonitrile in distilled water containing 0.1% TFA).

(Su *et al.*, 2022)



**Fig. 11.** Identification of molecular mass and amino acid sequence of the purified peptide (fraction A4) by LC-MS/MS.

(Su *et al.*, 2022)

**Table 1.** ACE inhibitory peptide sequences in the WSP from *P.pseudolinearis* and identification of the peptide sources from in silico digestion of *P.pulchra* plastid proteins.

FN	Peptide	Number of Peptides in Chloroplast Proteins	In Silico Thermolysin Digestion	
			○ <sup>a</sup>	× <sup>b</sup>
3	AYR	6	rbcL	apcE, atpA, cpcG, psaL, rps7
4	MTFF	0	- <sup>c</sup>	-
5	VRFK <sup>d</sup>	1	gltB	-
6	KFR	4	-	accA, cemA, chlI, rpl16
6	WHKPA	0	-	-
6	FFKWEF	0	-	-
7	FGGR	1	petJ	-
7	LVER	0	-	-
8	YRD	7	-	cpeA, moeB, odpA, orf148, rps14, ycf3, ycf21
8	FFR <sup>d</sup>	4	psaB, ycf22	psbT, ycf63
9	ARY	6	atpI, chlB, gltB	apcA, orf263, ycf46
9	RFR	3	-	odpA, rpoC1 (2) <sup>e</sup>
10	FAR <sup>d</sup>	7	clpC, orf174, ycf26	atpI, chlB, cpeA, ilvH
10	YLR	12	-	accA, apcA, apcB, apcD, apcE, apcF, carA, cpcA, orf114, rpl3, rpl19, rps9
10	VYRT	1	-	cpeA
10	FVCG	0	-	-
11	FFLREF	0	-	-
12	SRGL	1	-	rpl23
13	ACWR	0	-	-
13	RFAPR	0	-	-
14	ACPSGT	0	-	-
16	WER	1	-	psbA
17	LDY	17	chlN, cpcA, cpeA, infB, rbcL, ycf16	accA, apcA, apcB, atpA, infC, orf111, orf565, rpoA, rps1, syh, ycf24
17	LLEER	0	-	-
18	PGCRR	0	-	-
18	FLWWLR	0	-	-
20	AAGRFP	1	-	cpeA
23	LVFFGH	0	-	-
24	PVAFN	0	-	-
25	LRM <sup>d</sup>	2	cpcA	psbB
26	LRY	8	apcB, apcE, apcF, cpcB, cpeB	apcD, apcE, rbcR
27	CPSNN	0	-	-
27	AWRRP	0	-	-
27	LWT	4	trpG	psaB, psbD, psbZ
28	YRF	3	-	cpcG, odpA, syh
28	FRV <sup>d</sup>	8	petB, rbcL, rps5, rpoB (2), ycf26	pgmA, rpoB

(Kumagai *et al.*, 2021)

**Table 1. Cont.**

FN	Peptide	Number of Peptides in Chloroplast Proteins	In Silico Thermolysin Digestion	
			○ <sup>a</sup>	× <sup>b</sup>
29	VNLF	1	-	ycf38
29	PGDTY	0	-	-
30	EWYPH	0	-	-
32	KTFPY	0	-	-
34	FGRP-F <sup>d</sup>	1	rbcL	-
36	VESR	2	-	rbcL, rpoA
Total		101 peptides in 66 kinds of proteins (31 peptides were produced by in silico digestion)		

Data from *Pyropia pulchra* plastid (NC\_029861.1). <sup>a</sup> The peptide is produced by thermolysin digestion from the protein. <sup>b</sup> The peptide is confirmed in the protein sequence, but it is not produced by thermolysin digestion. <sup>c</sup> “-” not detected in the plastid genome. <sup>d</sup> The peptides are hydrolyzed by in silico thermolysin digestion. Hydrolysis position of peptides show “-” : VR-FK;LR-M;FR-V;FGRP-F;F-FR;F-AR. <sup>e</sup> The parentheses indicate number of in silico thermolysin digestion site in proteins.

(Kumagai *et al.*, 2021)



**Table 2.** ACE inhibitory activity of the synthetic peptide.

Peptide	IC <sub>50</sub> (μmol)	Reference
ARY	1.3	This study     <a href="#">[35]</a>
YLR	5.8	
LRM	0.15	
VYRT	0.14	
LDY	6.1	
FEQWAS	>2.8	
LRY	0.044	

**Table 3.** Amino acid composition of C-phycoerythrin (C-PC) and allophycoerythrin (APC) subunits from SP.

Amino acid	Amino acid composition (%)			
	C-PC- $\alpha$ subunit	C-PC- $\beta$ subunit	APC- $\alpha$ subunit	APC- $\beta$ subunit
Ala	14.80	16.90	11.20	13.70
Arg	4.30	6.40	7.50	5.60
Asn	3.70	4.10	1.90	3.70
Asp	5.60	5.80	5.00	6.20
Cys	1.20	2.30	0.60	1.20
Gln	4.30	2.90	1.20	3.10
Glu	4.90	4.70	9.30	3.70
Gly	8.00	6.40	9.90	8.10
His	0.60	0.00	0.00	0.00
Ile	6.80	5.20	7.50	7.50
Leu	8.00	8.10	6.80	8.70
Lys	5.60	2.30	3.70	5.00
Met	2.50	3.50	3.70	3.10
Phe	3.10	2.90	1.90	1.20
Pro	3.10	2.90	3.10	1.90
Ser	7.40	9.30	6.80	6.80
Thr	6.20	5.80	6.20	6.80
Trp	0.60	0.00	0.00	0.00
Tyr	6.80	2.90	5.00	7.50
Val	2.50	7.60	8.70	6.20
Total hydrophobic amino acids	33.40	39.00	36.10	33.60
Total essential amino acids	35.90	35.40	38.50	38.50

(Zhang *et al.*, 2022)

**Table 4.** Information of eight identified peptides from SP.

Peptide sequence	No. of amino acid residues	Theoretical mass (Da)	Observed molecular ion, $m/z$ (charge)	Toxin prediction
LDY	3	409.18	410.1915	Non-toxin
FDY	3	443.17	444.1766	Non-toxin
LRDMEI	6	775.39	776.3920	Non-toxin
IDA	3	317.16	318.1797	Non-toxin
LRETY	5	680.35	681.3540	Non-toxin
IQK	3	387.25	388.2538	Non-toxin
VTY	3	381.19	382.1962	Non-toxin
LGVP	4	384.24	385.2436	Non-toxin

(Zhang *et al.*, 2022)

**Table 5.** *In vitro* ACE-inhibitory activity of GTN4H protein hydrolysate following fast protein liquid purification (fractions A, B, and C) and further purification of fraction A by RP-HPLC (fractions A1-A5).

Fraction	ACE Inhibition (%)
A	69.74 ± 0.80
B	24.04 ± 0.45
C	59.95 ± 1.29
A1	N.D.
A2	N.D.
A3	N.D.
A4	95.4 ± 0.6
A5	5.00 ± 1.8

N.D.: not detectable.

(Su *et al.*, 2022)