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Binding of Captopril and Bioactive Tripeptides Val-Pro-Pro and Ile-Pro-Pro to Angiotensin I-Converting Enzyme (ACE I): Insights from DFT

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Introduction

Hypertension, or high blood pressure, is one of the most important risk factors for cardiovascular disease and death. It is a common condition that will catch up with most people who live into older age. Angiotensin-I-converting enzyme *(*ACE) inhibitors (Captopril, Enalapril, Lisinopril, Benazepril, Fosinopril, etc.) are a major class of antihypertensive drugs - they are commonly prescribed to treat high blood pressure and related conditions. ACE belongs to the group of zinc peptidases with HEXXH sequence segment or zincins, subdivision gluzincins (with motif His-Glu-Xaa-Xaa-His, Xaa stands for any amino acid) [1,2]. In the HEXXH motif the two His residues, together with the *C*-terminus Glu, ligate the essential zinc atom, and the Glu in the HEXXH motif has a catalytic function [3]. Possible alternative therapies for treatment of (pre)hypertension are nutraceuticals and functional foods that claim some physiological benefits. For example, food-derived proteins release variety of bioactive peptides which are similar in structure to peptide sequences acting in the organism and therefore can modulate their physiological functions. It emerges that milk-derived peptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) exert mild inhibitory activity against ACE [4,5]. These peptides have been shown to decrease blood pressure in hypertensive subjects [6] and are expected to play a vital role in the prevention of hypertension and hypertension-related disorders [7,8]. The aim of our *in silico* (DFT) study is to model the binding of milk-derived bioactive tripeptides Val-Pro-Pro and Ile-Pro-Pro and of the pharmaceutical drug Captopril (Figure 1) to the Zn^{2+}-HEXXH metal-binding motif of ACE I.

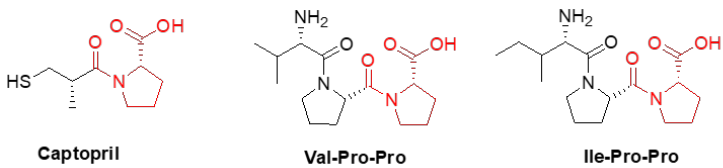

Fig. 1. Molecular structure of Captopril, Val-Pro-Pro and Ile-Pro-Pro.

Results and Discussion

In answering the question which inhibitor (drug or tripeptide) binds more readily to the HEXXH motif, we determined how strong the modeled tetra/penta-coordinated HEXXH motif interacts with the monosodium salts of the inhibitors by examining the change in the Gibbs energy (ΔG) in the following reactions:

$$[\text{Zn-2im-1ac-1w}]^{1+} + \text{captopril-Na}^0 \rightarrow [\text{Zn-2im-1ac-captopril-Na}]^{1+} + H_2O \quad (1)$$

$$[\text{Zn-2im-1ac-1w}]^{1+} + \text{vpp-Na}^0 \rightarrow [\text{Zn-2im-1ac-vpp-Na}]^{1+} + H_2O \quad (2)$$

$$[\text{Zn-2im-1ac-1w}]^{1+} + \text{ipp-Na}^0 \rightarrow [\text{Zn-2im-1ac-ipp-Na}]^{1+} + H_2O \quad (3)$$

To simplify the system, we use models for the amino acid residues that interact with metal cation(s) in the studied centers in a direct manner. The following models were considered (in accordance to the pK_a values of the amino acid side chains [9,10]) for the purpose of the current study: the side chains of usually deprotonated Asp/Glu were modeled as acetates (CH_3COO^-), whereas those of the neutral His were represented by methylimidazole ($C_4N_2H_6$).

B3LYP/6-31+G(3d,p) level of theory was chosen for all molecular electronic structure calculations in the gas phase, ε=1, and in protein environment, ε=4. This functional/basis set combination has been recently shown to be reliable in studying the function of the zinc cation in two centers of rhodopsin [11]. Monosodium salts of Captopril, Val-Pro-Pro and Ile-Pro-Pro inhibitors were optimized and these structures were used to build up models of HEXXH motif bound to

inhibitor molecules after a displacement of a water molecule (Figure 2). The arrangement of the amino acid models was preserved in the binding site/inhibitor constructs.

The binding of Captopril with deprotonated sulfhydryl group (such a structure was used in the simulation of the docking interactions of captopril to ACE I [12]) is predicted to be highly favorable, characterized with negative $\Delta G^1/\Delta G^4$ values (-86.3 kcal mol^{-1} and -31.0 kcal mol^{-1}, respectively).

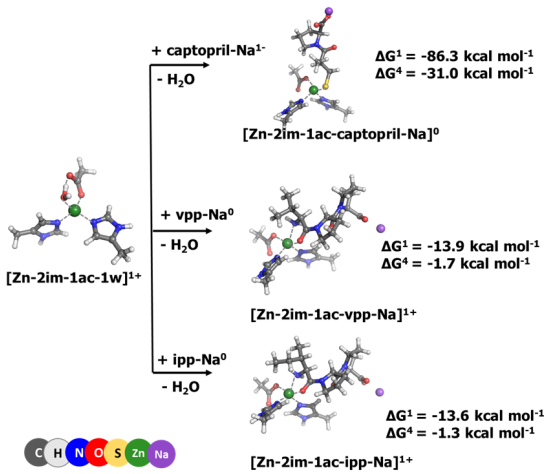

Fig. 2. Optimized geometries of the binding site model, $[Zn\text{-}2im\text{-}1ac\text{-}1w]^{1+}$, and binding site/inhibitor constructs. Gibbs energies of complex formation calculated for B3LYP/6-31+G(3d,p) optimized octahedral $[Zn\text{-}2im\text{-}1ac\text{-}captopril\text{-}Na]^{1+}$, $[Zn\text{-}2im\text{-}1ac\text{-}vpp\text{-}Na]^{1+}$ and $[Zn\text{-}2im\text{-}1ac\text{-}ipp\text{-}Na]^{1+}$ structures.

Val-Pro-Pro and Ile-Pro-Pro tripeptides coordinate to the metal cation via the valine's/isoleucine functional groups. Valine and isoleucine (along with leucine), are known as "branched chain amino acids" - they contain nonlinear aliphatic side chains (isopropyl and *sec*-butyl, respectively).

Valine NH_2 group plays a significant role in the binding site/inhibitor complex formation and influences the Val-Pro-Pro affinity towards Zn^{2+} binding site [13]. Similar effect can be supposed for the isoleucine NH_2 group. For Val-Pro-Pro the interactions with Zn^{2+}-HEXXH binding motif are predicted to be favorable in both the gas phase and protein environment, characterized with negative ΔG^1=-13.9 kcal mol^{-1} and ΔG^4=-1.7 kcal mol^{-1}.

For Ile-Pro-Pro the interactions with Zn^{2+}-HEXXH binding motif are predicted to be favorable in both the gas phase and protein environment, characterized with negative ΔG^1=-13.6 kcal mol^{-1} and ΔG^4=-1.3 kcal mol^{-1}.

The results obtained are in line with the tendency of Zn^{2+} to form complexes readily with S-donor ligands (Captopril). The interactions of Val-Pro-Pro and Ile-Pro-Pro with Zn^{2+}-HEXXH binding motif are expected to be favorable in both the gas phase and protein environment, but the ΔG values are indicative for a much lower activity of the tripeptide (compared to the drug molecule). Val-Pro-Pro and Ile-Pro-Pro do not differ significantly in their affinity for the Zn^{2+}-HEXXH binding motif.

Acknowledgments

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