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Bioinspired Interactions of Zn (II) and Cu (II) Metal Ions with the Antimicrobial Peptide Holothuroidin: Discovering the Action Mechanism

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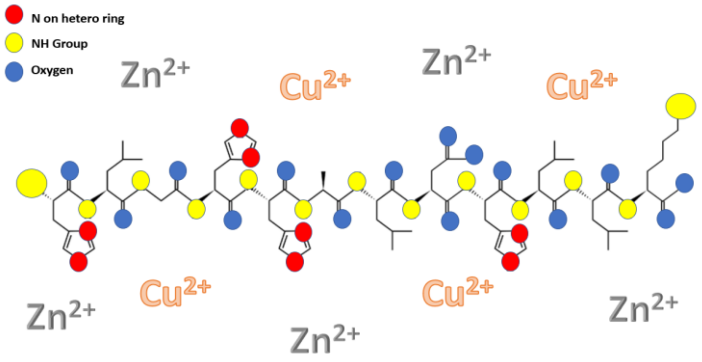
Introduction

The dramatic increase in antimicrobial resistance has led to active research for new treatments. Antimicrobial peptides (AMPs) are small 7 to 10 amino acids [1, 2]. One discovered mode of action of AMPs is membrane disruptive activity, associated with the interaction with membrane phospholipids [3]. Another way of action of AMPs is intracellular targeting. Some AMPs can cross the cell barriers and reach targets in the cytoplasm [2]. For some AMPs, the occurrence of bivalent metal ions (such as Zn^{2+} and Cu^{2+}) affects their activity or mode of action either because of binding metal ions, so that microbes cannot get enough nutrients essential for their life and virulence [4] or because AMPs need the metal ion as a booster of their antimicrobial activity, affecting the charge or the structure of the peptide [5]. Here, we discuss the action mechanism of Holothuroidin I (HLGHHALDHLLK) [6], a natural derived peptide from Mediterranean Sea cucumber *Holothuria tubulos*a, of the interactions with important metal ions located in mammals (such as Zn $^{2+}$ and Cu $^{2+}$).

Zinc (II), is the second most abundant transition metal in living organisms, [7] and present in superoxide dismutases, central enzymes in bacteria and fungi, associated with the detoxification of ROS generated by host cells during host-pathogen interactions [8]. Copper is another essential metal ion, which can switch between oxidized (Cu^{2+}) and reduced (Cu^{+}) states. It is essential for enzymes involved in cellular respiration, iron transport, and superoxide dismutation. Besides, the redox activity of copper may also contribute to its toxicity to microbes (and mammals) through classic copper-catalyzed Fenton chemistry and an increase in reactive oxygen species [9].

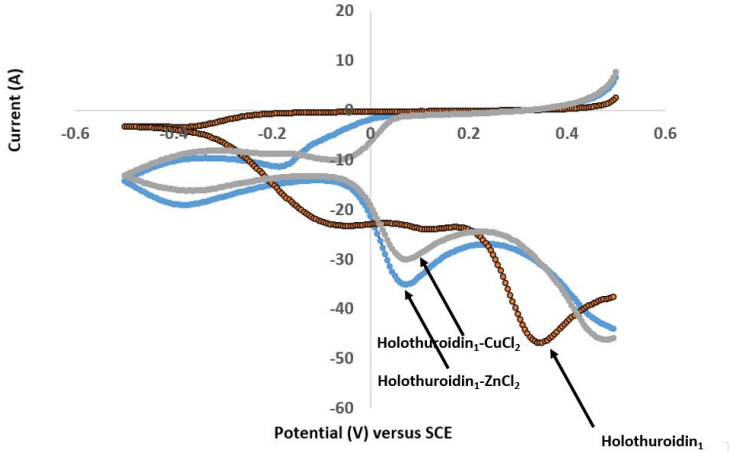
The action mechanism evaluation was performed using potentiometric titrations, conductivity studies, UV-Vis studies, voltametric methods, and density function theory studies, giving valuable inside into the mechanism of interactions of Holothuroidin I with copper and zinc.

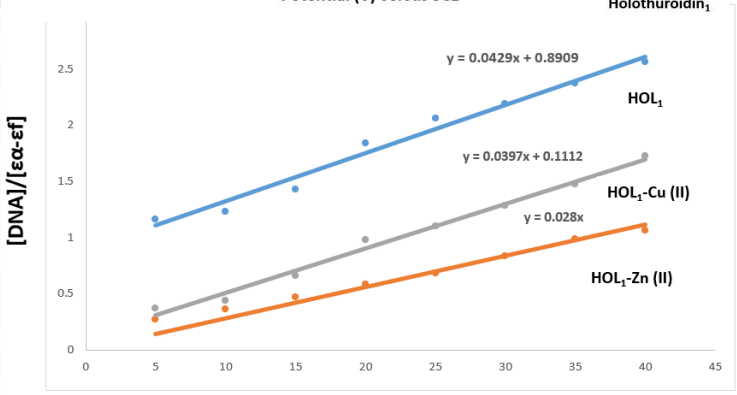


Physicochemical Characteristics	HOL_1 - Zn (II)	HOL_1 - Cu (II)
Stretch	0.2899	0.4522
Bend	72.4009	20.1957
Stretch-Bend	-0.1613	0.0075
Torsion	-13.2517	-18.0636
Non-1,4 VDW	-6.0180	-9.3509
1,4 VDW	18.9466	15.0045
Charge/Charge	88.6057	88.6599
Charge/Dipole	-56.9095	-65.9070
Dipole/Dipole	4.6666	6.2357
Total Energy (kcal/mol)	108.5692	37.2341
Geometry	Trigonal Planar	Tetrahedral
LUMO (eV)	-8.668	-8.175
HOMO (eV)	-9.282	-8.765
Δ_{Gap} (eV)	0.614	0.59

Methods

In order to evaluate the binding mechanism of the metal ions with the peptide, we performed electrochemical studies (cyclic voltammetry, conductivity and potentiometry). For the evaluation of the peptide metal complexes activity on DNA base pairs we performed UV-Vis experiments. Finally, the density functional theory studies gave us an inside on the metal centre geometry of the complex formation.





Results

Having a rich donor atom environment, HoL_1 peptide, interacts with metal ions such as Cu (II) and Zn (II), that are responsible for human body homeostasis. The results indicated that this peptide could change physiology both for humans and microbes in the body. It is known that copper (II) ions forms tetrahedral geometry with the peptide, while the zinc metal ions form trigonal planar geometries based on our calculated results. Additionally, based on our electrochemical experiments we have concluded that zinc affects and alters the peptide structure much more than the copper which means that this will have an impact in Zn related biochemistry processes like cellular signalling. Moreover, when HOL1 interacts with zinc is able to interact stronger with DNA.

Metal Centre Geometry of HOL_1 - Zn (II)	Length (Å)
Zn (31) - Cl (33)	2.2465
Zn (31) - Cl (32)	2.2471
O (29) - Zn (31)	1.8996
N (30) - Zn (31)	1.9263
O (14) - Zn (31)	1.8953
N (15) - Zn (31)	1.9312
Metal Centre Geometry of HOL_1 – Cu (II)	Length (Å)
Cu (31) - Cl (33)	2.1703
Cu (31) - Cl (32)	2.1703
Cu (31) - O (14)	1.8182
O (29) – Cu (31)	1.8163
Cu (31) – N (15)	1.8523
N (30) – Cu (31)	1.8532
Metal Centre Geometry of HOL_1 - Zn (II)	Angle (Degree)
Cl (33) - Zn (31) - Cl (32)	90.6152
Cl (33) - Zn (31) - O (29)	172.0257
O (29) - Zn (31) - N (30)	84.6158
O (29) - Zn (31) - O (14)	84.5623
N (30) - Zn (31) - O (14)	166.6899
N (30) - Zn (31) - N (15)	92.1298
Metal Centre Geometry of HOL_1 - Cu (II)	Angle (Degree)
Cl (33) - Cu (31) - Cl (32)	93.5325
Cl (33) - Cu (31) - O (14)	87.0171
O (14) - Cu (31) - O (29)	91.3399
O (14) - Cu (31) - N (15)	87.5763
O (29) - Cu (31) - N (30)	81.9590
N (15) - Cu (31) - N (30)	96.2893

Conclusions

The interactions between zinc and copper metal ions with the natural derived peptide HOL_1, have been investigated showing that the rich donor environment of the peptide forms complex formations with the metals. Stronger formations occurred between the peptide and zinc making it able to alter physiological processes. The thermodynamic profile of the interactionn is something that it is ongoing in our lab.

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