DOI: http://dx.medra.org/10.17374/CI.2022.104.6.46

Adriana Miller
Faculty of Chemistry, University of Wroclaw (Poland)
adriana.miller@chem.uni.wroc.pl

INVESTIGATION OF THE INTERACTIONS BETWEEN METAL IONS AND ANTIMICROBIAL PEPTIDES FROM CLAVANIN AND SAAP FAMILY

Antimicrobial peptides play an important role in defense against pathogens in many organisms. Currently, they are considered potential candidates for new therapeutics. Metal ions such as Zn(II) or Cu(II) can influence their biological activity. Herein, the investigation results of 8 peptides and their Zn(II) and Cu(II) complexes are discussed.

Introduction

Antimicrobial peptides (AMPs) are small biologically active molecules, present in almost all multicellular organisms. They are a part of their innate immune system and play an important role in defense against pathogens **[1, 2]**. Currently, over 3,400 AMPs have been isolated and described in specific databases **[3]**. This group of compounds is very diverse and shows a variety of lengths (7-100 amino acids), secondary structures (α-helix, β-sheet, random coil, etc.) and modes of action (disrupting the surface of the pathogen or achieving intracellular targets). Most of them are α-helical cationic peptides **[2, 3]**. Knowing that bacterial pathogens have negatively charged cell membranes, positive charge facilitates the interaction between the peptide and the pathogens' surface. In addition to antibacterial activity, they can also show anti-cancer, antiviral and antifungal properties **[2, 3]**. Antimicrobial peptides are also known for inducing negligible resistance in microorganisms, including drug-resistant ones. They owe it, inter alia, to using multiple mechanisms of action simultaneously or to non-specific mechanisms, to which resistance is difficult to develop **[2]**. This feature is particularly important nowadays when the problem of drug-resistant pathogens is still increasing and new therapeutics are intensively sought **[2]**.

Some AMPs can interact with biologically relevant metal ions, such as Zn(II) or Cu(II). These metals are crucial for the proper function of many enzymes, necessary for the survival of both host and pathogen **[1]**. Because of their significant meaning, some organisms have developed a defensive mechanism called nutritional immunity. It involves the capture of free metal ions by specific molecules, to prevent their uptake by pathogens **[4]**. Interestingly, antimicrobial peptides can be engaged in this process and play the role of capturing agents. Moreover, metal ions can affect the AMPs' secondary structure or charge, which can result in enhanced antimicrobial activity **[1]**.

The Fernando Pulidori Prize (15th Edition) was awarded to Adriana Miller during the 2022 edition of the International Symposium on Metal Complexes (ISMEC 2022) held in Valencia (Spain), from 5th to 8th of June 2022.

All this information was an inspiration to find and understand the relationship between coordination chemistry, structure, thermodynamics and mode of action of Zn(II) and Cu(II) complexes of chosen antimicrobial peptides. In this work, the results obtained for eight peptides from two families are discussed. For the first family, clavanins, an interesting relationship between spatial arrangement, coordination of metal ions and biological activity was observed. The second family, SAAPs, was particularly important from the bioinorganic chemistry point of view.

Fig. 1 - Amino acid sequence of clavanin A, B, C, D and E. The differences between peptides are highlighted [8]

Deeper knowledge and understanding of metal-peptide interactions can be a precious contribution to the bioinorganic chemistry of peptides and enable to use peptides as matrixes in designing new selective drugs. Due to the possible therapeutic meaning, the particular focus was put on the results obtained at pH 7.4.

Clavanins

Clavanins were originally isolated from the sea squirts *Styela clava*. This group of peptides has six members: clavanin A, B, C, D, E and clavaspirin **[5, 6]**. They are α-helical cationic peptides built from 23 amino acids **[6]**. Because of their high content of histidines, it was expected that they would be able to bind biologically essential metal ions such as Zn(II) or Cu(II). So far, clavanin A and C are the most studied in the literature. At pH 5.5 they act against *E. coli, L. monocytogenes, K. pneumoniae* and *C. albicans*. Moreover, the addition of Zn(II) ions significantly enhances the action of clavanin A against *E. coli* **[7, 8]**.

The thermodynamic studies of clavanin A-E (Fig. 1) complexes started with the mass spectrometry measurements, which clearly showed that all five examined peptides form mononuclear complexes with Zn(II) or Cu(II). Basing on the potentiometric titration, the stability constants for each complex were determined **[8]**. Moreover, thanks to the other methods (UV-vis, CD, NMR, DFT), the mode of coordination and geometry of the formed complexes were established. At pH 7.4, all clavanins coordinate Zn(II) by three nitrogens from imidazole groups from histidine side chains (His10, His11, His17 for clavanin A, B and E; His10, His11, His 21 for clavanin C and D) **[8]**.

Cu(II) complexes show square-planar geometry and are formed by the coordination of three imidazole nitrogens from histidine side chains and one amide nitrogen from the peptide main chain. The mode of coordination is different for clavanin C, due to the presence of the ATCUN motif (Amino Terminal Cu(II) and Ni(II) binding motif) **[9]**, where copper(II) ion is bound by four nitrogens (N-terminal amine group, two subsequent amide groups from the main peptide chain and the imidazole ring from His3 side chain) and forms a highly stable, square-planar complex **[10]**.

To compare the affinities of particular peptides to metal ions, competition diagrams were prepared. They are based on the data from potentiometric measurements and show the hypothetical situation when equimolar amounts of reagents are mixed. For Zn(II) complexes, these affinities are rather comparable, but for Cu(II) complexes at pH above 6, Cu(II)-clavanin C shows significantly higher stability than the others, which is caused by the presence of the ATCUN motif (Fig. 2).

From the antimicrobial activity point of view, Zn(II)-clavanin C complex was the most effective and versatile compound at pH 7.4. It was active against *E. coli, E. faecalis, S. aureus* and *C. albicans* **[8]**. DFT helped to explain this observation - despite presenting the same mode of coordination and even using the same donor groups, the bond

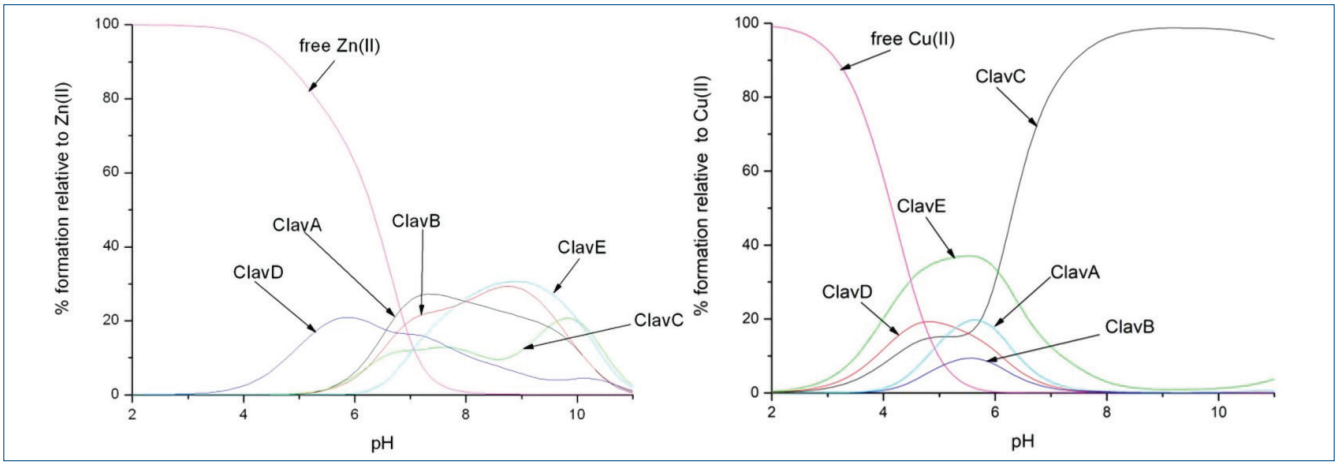

Fig. 2 - Competition plots between clavanin A-E and Zn(II) (left) or Cu(II) (right), describing complex formation at different pH values in a hypothetical situation, in which equimolar amounts of the six reagents are mixed [8]

length in Zn(II)-clavanin C is slightly larger than in other clavanins. This elongation is caused by prefolding of the peptide, which takes place before binding of the metal. Because of this prefolding, an amide group from the main chain is present in the close vicinity of the coordinated Zn(II) ion. Hydrogen from the mentioned amide group is situated exactly under the Zn(II) and slightly repels it from the coordination sphere, which is observed by the elongation of the bond and translates to weaker metal-peptide interaction and easier dissociation of Zn(II). Local accumulation of metal ions may be toxic to pathogens, which may explain the increased antimicrobial activity of the Zn(II)-clavanin C complex **[8]**. Such a small difference in structure, which brings so meaningful difference in the antimicrobial activity was described as the "chemical butterfly effect".

SAAP

SAAP (*surfactant-associated anionic peptides*) are naturally present in ovine pulmonary surfactant. They are short, Asp-rich peptides and their special feature is a negative charge (the vast majority of AMP peptides are cationic) (Fig. 3) **[10]**. Three members of this group: SAAP2, SAAP3 and SAAP6 are active mainly against *Mannheimia haemolytica*, which causes pneumonic mannheimiosis - a serious disease of the respiratory tract in sheep, cattle and goats **[10, 11]**. Interestingly, they show antimicrobial activity only in the presence of Zn(II) ions **[10]**.

All three peptides form complexes with zinc(II) in metal:ligand stoichiometry 1:1. Potentiometric titrations indicated that SAAP2, SAAP3 and SAAP6 coordinate Zn(II) ions by Asp residues, which are anchoring sites, and by *N*-terminal amine group. The affinity of all peptides for zinc(II) around pH 7.4 is comparable **[12]**.

Similarly to zinc(II), copper(II) binds to all three peptides in a 1:1 stoichiometry. To determine the mode of coordination, both potentiometric and spectroscopic methods (UV-Vis, CD, EPR) were used. SAAP2 and SAAP3 bind Cu(II) by *N*-terminal amine group - as an anchoring site, carboxylates from the Asp residues in the first/second position and by amide nitrogens. SAAP6, which lacks Asp residue in the first and second position, anchors copper(II) by *N*-terminal amine and then coordinates only by amide nitrogens. Despite engaging the amide group in SAAP6 at significantly lower pH, this peptide has also lower affinity for Cu(II) ions. As shown in the competition diagram (Fig. 4), the stability of Cu(II) complexes increases together with the number of Asp residues. This observation can be explained by the presence of carboxylates in the first and/or second positions, which modulate Lewis acidity for competition with amide protons and its peptide affinity **[12]**.

SAAP2: **GDDDDDD**

SAAP3: **DDDDDDD**

SAAP6: **GADDDDD**

Fig. 3 - Amino acid sequences of SAAP2, SAAP3, and SAAP6 peptides

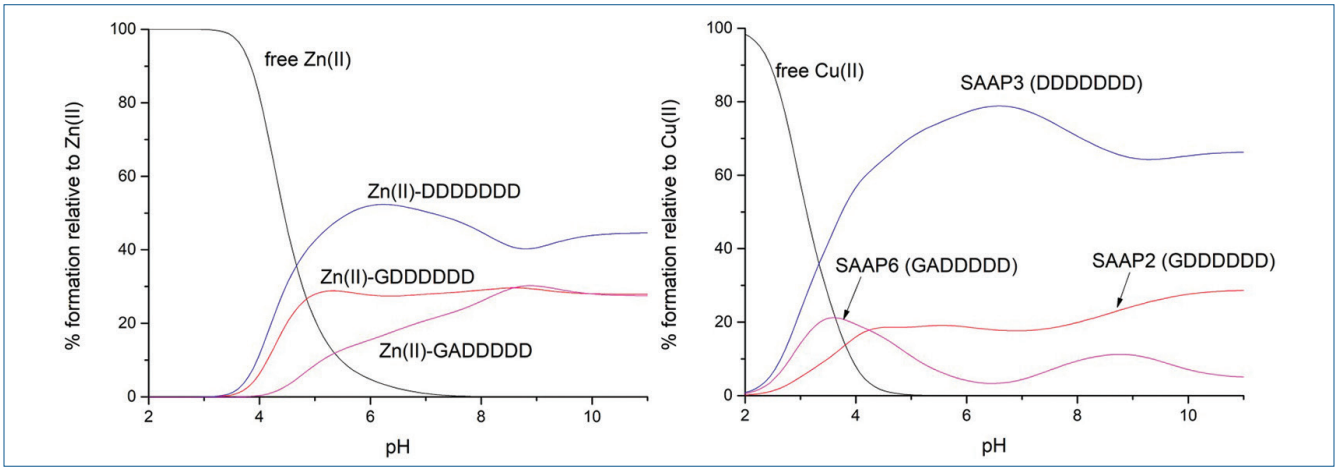

Fig. 4 - Competition plots for SAAP2, SAAP3, SAAP6 and Zn(II) (A) or Cu(II) (B), showing relative the amount of each complex at different pH values for the hypothetical situation in which equimolar amounts of the four species are mixed [12]

Conclusions

Because of the significant meaning of the antimicrobial peptides - not only as biologically active molecules but also as interesting objects from the point of view of bioinorganic chemistry - the studies on their Zn(II) and Cu(II) complexes can bring precious information, important on several levels. It can make a large contribution to the general knowledge of the basic bioinorganic chemistry of Zn(II) and Cu(II), while it describes different binding sites, thermodynamical features and structural details. Moreover, it can also be a step towards finding or designing new specific antimicrobial therapeutics.

Acknowledgements

The above research was carried out as part of the National Science Center project "Antimicrobial peptide-metal interactions - understanding the relationship between coordination chemistry, structure, thermodynamics and mode of action" (UMO-2017/26/E/ST5/00364), headed by my supervisor, Prof. Magdalena Rowińska-Żyrek. She has always been a support for me and willingly shared her knowledge and enthusiasm, for which I am very grateful. I would also like to thank Dr. Agnieszka Matera-Witkiewicz and M.Sc. Aleksandra Mikołajczyk for all support during my work at antimicrobial activity assays carried out at the Medical University in Wroclaw.

References

[1] J.L. Alexander, Z. Thompson *et al., ACS Chem. Biol.*, 2018, **13**, 844.

[2] D. Łoboda, H. Kozłowski *et al., New J. Chem.*, 2018, **42**, 7560.

[3] G. Wang, X. Li *et al., Nucleic Acids Res.*, 2016, **44**, 1087.

[4] J. Wątły, S. Potocki *et al., Chem. Eur. J.*, 2016, **22**, 15992.

[5] R.I. Lehrer, J.A. Tincu *et al., Integr. Comp. Biol.*, 2003, **43**, 313.

[6] I.H. Lee, C. Zhao *et al., FEBS Letters*, 1997, **400**, 158.

[7] S.A. Juliano, S. Pierce *et al., Biochemistry*, 2017, **56**, 1403.

[8] A. Miller, A. Matera-Witkiewicz *et al., Inorg. Chem.*, 2021, **60**, 12730.

[9] C. Harford, B. Sarkar, *Acc. of Chem. Res.*, 1997, **30**(3), 123.

[10] K.A. Brogden, A.J. de Lucca *et al., Proc. Natl. Acad. Sci. U.S.A.*, 1996, **93**, 412.

[11] T.E. Besser, E.F. Cassirer *et al., Prev. Vet. Med.*, 2013, **108**, 85.

[12] A. Miller, A. Matera-Witkiewicz *et al., IJMS*, 2021, **22**(13), 6971.

Studio delle interazioni tra ioni metallici e peptidi antimicrobici derivanti dalla clavanina e dalla famiglia SAAP

I peptidi antimicrobici svolgono un ruolo importante nella difesa contro i patogeni in molti organismi. Attualmente, sono considerati potenziali candidati per nuove terapie. Gli ioni metallici come Zn(II) o Cu(II) possono influenzare la loro attività biologica. Qui vengono discussi i risultati dello studio su 8 peptidi e dei loro complessi Zn(II) e Cu(II).