2023

Quantifying Metal Interactions with the Antimicrobial Peptide Calcitermin

Maria G. Aviles-Sandoval
University of San Francisco, mgavilessandoval@dons.usfca.edu

Michael Stevenson
University of San Francisco, mstevenson3@usfca.edu

Quantifying Metal Interactions with the Antimicrobial Peptide Calcitermin

Maria Aviles-Sandoval, Marcela Valle, Sohee Choi, Michael J. Stevenson

Department of Chemistry, University of San Francisco, 2130 Fulton St., San Francisco, CA

Abstract

Antimicrobial Peptides (AMPs) are novel therapeutic agents that play important roles in the innate immune system with its ability to kill gram-positive and gram-negative bacteria. Antibiotics are losing their efficacy, thus requiring a larger dose of medicine for treatment resulting in the increase of antibiotic resistance. AMPs are an attractive approach for exploration due to their broad-spectrum activity and ease of synthesis. Furthermore, fewer bacteria have developed resistance to AMPs. Several AMPs have demonstrated increased antimicrobial activity with metal ions, like Zn(II) and Cu(II) binding to the peptide. In this study, we performed the purification of Calcitermin through high-performance liquid chromatography (HPLC) and will be analyzing the metal-binding thermodynamics through isothermal titration calorimetry (ITC).

Antimicrobial Peptides (AMPs)

Antibiotic resistance is a complex issue driven from the overuse and misuse of prescriptions. Unlike traditional antibiotics, antimicrobial peptides have different modes of action as illustrated in Figure 1, binding of metal ions creates the formation of reactive oxygen species (ROS) that are toxic to pathogens, disrupts the cell membrane leading to cell death and reduces metal availability essentially starving the pathogens.

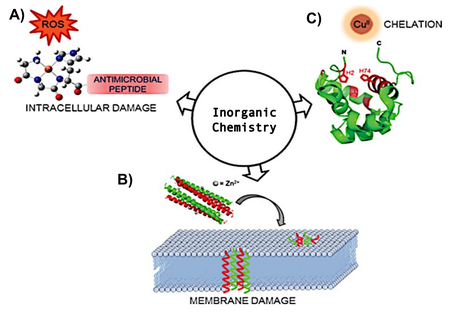


Figure 1. Antimicrobial peptide metal related modes of action.[1]

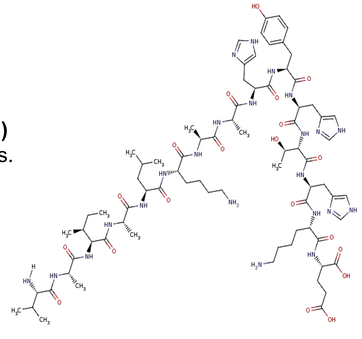


Figure 2. Molecular structure of Calcitermin.[2]

Calcitermin is a 15-residue AMP **(VAIALKAAHYHTHKE)** found in human airways. Calcitermin is a cleavage product of S100 Calgranulin C. In acidic conditions, Calcitermin develops a cationic charge with its three protonated histidines enhancing its interaction with negatively charged bacterial membranes. It is believed that Calcitermin binds to Zn(II) and Cu(II) at the HxHxH motif. This motif plays a critical role in metal coordination by providing a high-affinity binding site for metal ions.

Motivation

A major public health concern is the rise of antibiotic resistant pathogens and an attractive alternative to antibiotics is antimicrobial peptides (AMPs). Previous research demonstrated Calcitermin has metal-binding capabilities with Zn(II) and Cu(II). Insight into the metal binding interactions with Calcitermin may lead to the development of new types of therapeutics to combat the rise of antimicrobial resistance.

Objectives & Hypothesis

- Understand the binding thermodynamics of Calcitermin with Zn(II) and Cu(II).
- Characterize structural changes that occur upon metal binding of Calcitermin using nuclear magnetic resonance (NMR).

We hypothesize that Cu(II) will bind to Calcitermin with a higher affinity than Zn(II), but that Zn(II) will be able to compete with Cu(II) at the HxHxH motif.

Solid-Phase Peptide Synthesis (SPPS)

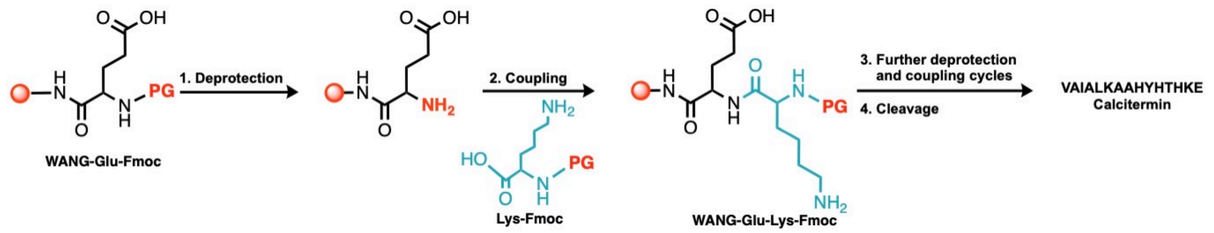


Figure 3. Steps of Solid-Phase Peptide Synthesis (SPPS).

The solid-phase peptide synthesis (SPPS) method was employed, with WANG resin serving as the solid support for the peptide chain assembly. Synthesis was conducted from C-terminus to N-terminus. To protect the N-terminus during synthesis, the Fmoc group was utilized as a temporary protecting group (red bead). Washing and swelling steps were conducted using dimethylformamide (DMF) to activate the resin and remove impurities effectively. For deprotection of the Fmoc group, 20-25% 4-methylpiperidine was employed, allowing the N-terminus to be exposed for subsequent coupling reactions. The coupling agent N,N-diisopropylethylamine (DIEA) (20 eq. ratio) was used to facilitate peptide bond formation. Finally, the fully synthesized peptide was cleaved from the resin using dichloromethane (DCM), resulting in the release of Calcitermin **(VAIALKAAHYHTHKE)**.

Purification & Mass Spectrometry

Purification was conducted through high-performance liquid chromatography (HPLC). Approximately 0.0035g of the peptide was dissolved in 10:90 MeOH:water with 0.1% formic acid (FA). Calcitermin was monitored at 280 nm. The liquid sample was then injected into a reverse-phase C18 HPLC column. Solvent A (MeOH with 0.1% FA) and Solvent B (water with 0.1% FA) were used with Solvent B being held constant at 10% from 0-5 minutes and increased from 10% to 50% over 45 minutes then 50% to 100% for the remaining 5 minutes. Aliquots from HPLC were prepared with 50 µL sample and 950 µL of solvent and confirmed through electrospray ionization mass spectrometry (ESI-MS).

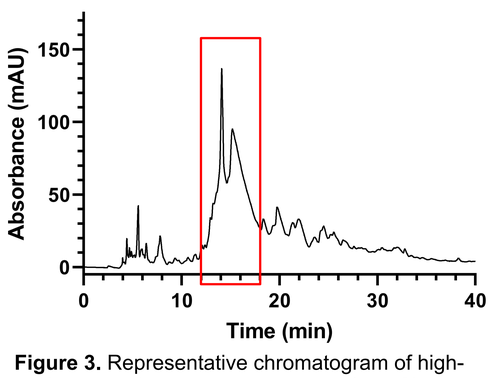


Figure 3. Representative chromatogram of high-performance liquid chromatography (HPLC). Peaks observed at 14.12 min and 15.19 min were analyzed on ESI-MS.

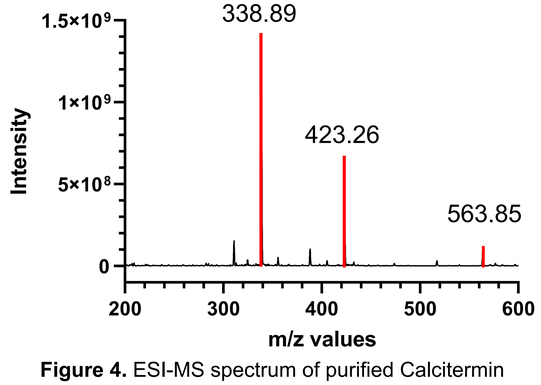


Figure 4. ESI-MS spectrum of purified Calcitermin demonstrating expected peaks of 338.89, 423.26, and 563.85. Peaks at 310 and 388 were identified as solvent peaks.

Isothermal Titration Calorimetry (ITC)

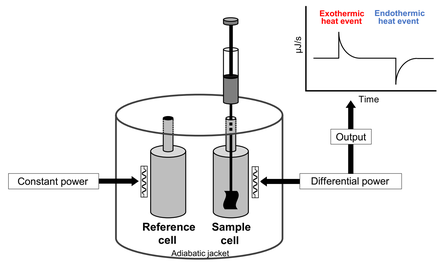
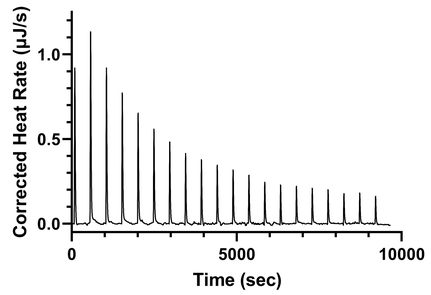


Figure 5. Schematic depicting the components of ITC.



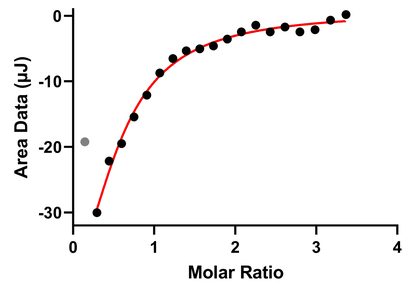


Figure 6. Representative thermogram of 1.00 mM Cu(II) titrated into 0.100 mM Calcitermin in 50 mM Bis-Tris buffer at pH 7.4. The one-set-of-sites independent fitting model for each titration is shown by the red line: Bis-Tris, n = 0.488, K_{ITC} = 2.5 x 10^4 ΔH_{ITC} = -29.58 kJ/mol. The datum in grey is excluded from the fit.

Conclusion & Future Work

We have successfully synthesized and purified Calcitermin. We will continue to perform isothermal titration calorimetry (ITC) with various buffer systems to quantify and corroborate Zn(II) and Cu(II) metal-binding thermodynamics to Calcitermin. Additionally, we will characterize the metal binding site on Calcitermin through NMR spectroscopy and electronic absorption spectroscopy.

Acknowledgements & References

Thank you to the USF Chemistry Department, Dr. Michael Stevenson, and to the members of the Stevenson Lab: Tiffany Alvarez, Jaden Lucas-Retherford, Marcela Valle, Keana Davis and Nicole Colligan. Thank you to Dr. Ron Najafi at Emery Pharma for Summer Research Funding.

References