STRESZCZENIE W JĘZYKU ANGIELSKIM

In the recent years, the problem of antimicrobial resistance has become one of the most serious global health threats. Treatment of bacterial and fungal infections with conventional antimicrobial drugs becomes ineffective, therefore novel, effective treatments are being actively sought. One of the promising groups of compounds are antimicrobial peptides (AMPs). Because of the general lack of resistance towards them, AMPs are being relied on as novel class of therapeutics. AMPs are small, mostly cationic peptides that take part in the non-specific immune response shared by all kingdoms of organisms. Different AMPs may be active, among others, against bacteria, fungi, viruses or parasites. There are several ways in which AMPs can affect pathogen cells including cell membrane disruption by formation of pores or membrane channels, production of reactive oxygen species or inhibition of the cell wall, nucleic acids and proteins synthesis. For some AMPs, the presence of divalent metal ions has an effect on their activity or mode of action. AMPs can use the metals in two ways: (i) in a process called nutritional immunity, in which AMPs bind metal ion, so that there is insufficient amount for the survival and virulence of microbes or (ii) AMPs need metal ion as a booster of their antimicrobial activity, affecting the charge and/or structure of the peptide.

The aim of this study was to understand the bioinorganic chemistry of Zn^{2+} and Cu^{2+} complexes with selected antimicrobial peptides and verification whether and how the presence of metal ions can determine their antimicrobial activity. Four groups of AMP were selected for the study: (i) alloferon 1 and alloferon 2, (ii) calcitermin and its mutants, (iii) proteolytic fragments of semenogelins and their mutual fragment, and (iv) amylin derivatives - rat amylin and pramlintide. In order to perform physicochemical and thermodynamic characterization of the studied ligands and their complexes, a number of experimental techniques were used: mass spectrometry, potentiometric titrations, UV-Vis, CD and NMR spectroscopy. Moreover, in cooperation with two research centers in Wrocław, biological studies (dr Agnieszka Matera-Witkiewicz, Medical University) and atomic force microscopy studies (dr hab. Joanna Olesiak-Bańska, Wrocław University of Science and Technology) were carried out. We identified the most probable metal ion binding sites, characterized donor atoms involved in the coordination in the wide pH range and determined geometry of the copper complexes. Comparison of the thermodynamic stability of all studied AMP complexes showed that the most effective coordination modes are: (i) histamine-like coordination - involving the N-terminal amino group and the histidine imidazole located in the first position in the sequence (ii) 4N coordination involving free N-terminal amino group and 3 imidazole nitrogens and (iii) 3N or 4N coordination mode via characteristic motifs (-HXXXH- or HXH… HH… HH) which are present in some studied ligands. Moreover, we demonstrated very high biological activity of calcitermin and its mutants, which is most probably achieved through local charge or/and structure changes. Additionally we excluded the antimicrobial effect of alloferons and demonstrated antibacterial effect of semenogelins which is partially determined by the presence of metals. We have explained the mechanism of the antifungal properties of the Zn^{2+}-pramlintide complex. The characteristic change (bending) of the backbone structure initiates fibrill formation, which probably disrupt fungal cell in a needle-like way. The demonstrated antimicrobial activity does not usually correlate with the thermodynamic stability of the investigated complexes. In the future the most promising AMPs will serve as scaffolds for a rational design of novel metal-AMP complexes with enhanced features which we think contribute to the antimicrobial efficacy of these complexes.