Abstract

This PhD project is focused on the development of new polymeric materials with antibacterial and antifungal activity.

The first part of the work was dedicated to the synthesis and the insertion of a modified amino acid into an antimicrobial peptide (AMP), with the aim to retain the action mechanism on bacterial membranes. Understanding the mechanism of action of AMPs is important for the rational design of new drugs. For this reason, I have collected structural properties, antimicrobial activity values and biological origin of antimicrobial peptides from published data. I have calculated the most relevant chemical physical properties like charge, hydrophobic moment, helicity, flexibility, isoelectric point, Boman and instability index and penetration capabilities. This data collection work permitted us to create YADAMP (www.yadamp.unisa.it), a web database with detailed informations on AMPs. YADAMP database contains the highest number of active sequences with proven antimicrobial activity. YADAMP peremitted me to do a work of data mining that end up with the choice of a peptide, a defensine, to be used as template for developing a new photoresponsive peptide. The peptide, hereafter indicated as ALY, is a short α-helix, membrane active AMP with a tyrosine in the sequence. I have developed the modified analogue replacing the tyrosine by a modified tyrosine with azobenzene group in the side chain. The modified amino acid, named Fmoc-azoTyr, was synthesized according to the classic scheme of diazocopulation reactions. I have chosen the azobenzene group because it permits a reversible *trans* to *cis* photochemical isomerization. The photo-induced switch will, potentially, permit to turn on or off the peptide antimicrobial activity. As described in the following part, we have already confirmed the antimicrobial and antifungal activity of azobenzene group.

Therefore, the modified peptide might have a broader spectrum of activity due to the azobenzene presence, and it might act as a prodrug, generating *in vivo* the antimicrobial azo compound. I evaluated the difference in membrane permeability due to the introduction of the modified amino acid performing studies of membrane permeability during a period in the group of Prof. Peter Walde at the Swiss Federal Institute of Technology (ETH Zurich). Calcein leakage assays showed that the modified amino acid introduced into active sequence increased the membrane permeability of a suspension of giant unilamellar vesicles compared to the unmodified peptide at the same conditions. I have prepared large unilamellar vesicles formed by POPC/POPG (mixture 90:10 mol/mol), that contain HRPC enzyme, by mechanical extrusion and I performed measurements of enzyme leakage after interaction with the peptide. In this assay the membrane permeability was associated with an increase in enzyme activity. Preliminary results of enzyme leakage showed a greater membrane perturbation with time-dependence enzyme leakage from LUVs after interaction with modified peptide.

The second part of my research work was focused on the design and the synthesis of antimicrobial low molecular weight molecules with antimicrobial and antifungal activity. I used the phytoalexin resveratrol as template to design a new class of active compounds with azobenzene structure. I selected the best candidates by preliminary *in silico* test of ADMET properties and then I synthesized the azo compounds with lowest *in silico* toxicity values (A1, A2, A3, A4, A5, B10 and B11) according to the classic scheme of diazocopulation reaction. The antimicrobial activity and the thermal stability of each compound were evaluated. The majority of synthesized compounds exhibited high antibacterial activity against *S. aureus* and antifungal activity against *C. albicans*, but they were inactive against Gram-negative bacteria such as *P. aeruginosa* and *S. Typhimurium*. The different antibacterial activities of synthesized azo compounds suggest that these molecules interact with protein

receptors and that the interaction with membranes is of minor importance. To validate this hypothesis, I carried out structural modifications of azo compounds to enhance their biological activity. I have used the best antimicrobial azo compounds named A4 (4'-hydroxy-(4-hydroxy-3,5-dimethyl)-azobenzene) as lead compound to synthesize several analogues having modifications on the first and on the second azobenzene ring. This includes the moving of the phenolic hydroxyl group from *para*- to *meta*-position, the removal of the phenolic hydroxyl group, and the replacement of the phenolic hydroxyl group by the methoxy or the methyl group. In this way I obtained molecules with antibacterial and antifungal activity higher than lead compound.

The antimicrobial activities of these azo compounds and their thermal stability are very promising and indicate that these molecules may have interesting and therapeutically significant applications. A possible application is the insertion of azo compounds into polymeric matrices to produce composites with low production cost, good processability, and antimicrobial potential. Using solvent casting and mold casting methods, I realized active antimicrobial and antifungal films using polyolefins (such as PP and LLDPE) and biodegradable polymers (such as PLA, PVA and Mater-B), by introducing different percentages of antimicrobial azo dyes in polymer matrices. The obtained thin films retained the proprieties of the pure matrices without azo compounds such as thermal proprieties, flexibility and transparency; also I prepared transparent amorphous films, as confirmed by X-Ray analysis. The films exhibited antimicrobial activity and the capability to inhibit biofilms formation of *S. aureus* and *C. albicans*. Concentration of 0.01% (w/w) permitted the preparation of active, uncolored and transparent films. This is the first time that novel azobenzene based antimicrobial compounds have been added into polymer films. These preliminary tests confirmed that the new materials realized in this thesis are promising for future applications in the field where

an intrinsic antimicrobial ability of the material is required, like biomedical tools, antibacterial surfaces, and films for food packaging. Spectrophotometric investigation of the azo compound release from the polymer matrices is currently undergoing.