

Boc(β ala) $_2$ N $_2$ H $_3$ and its interaction with silverA. Holzheu¹, A. Crochet¹, K. M. Fromm^{1*}¹University of Fribourg

INTRODUCTION: For centuries it is known that silver possesses antimicrobial properties. It was regularly used for the treatment of burns, wounds and several bacterial infections, but with the emergence of antibiotics it was nearly forgotten for almost 50 years^{1,2}. Nowadays, due to the rising concern regarding infectious diseases induced by multidrug-resistant bacteria, silver has made a remarkable comeback as a potential antimicrobial agent². Therefore, we study the antimicrobial effects of a dipeptide, Boc(β ala) $_2$ N $_2$ H $_3$, that has four potential silver coordination sites and the capability, due to the hydrazine end group, to reduce silver ions to silver nanoparticles (AgNPs). **METHODS:** To obtain the Boc(β ala) $_2$ N $_2$ H $_3$ dipeptide, a standard liquid phase synthesis was used³. The dipeptide was characterized by ¹H- and ¹³C-NMR, MS-ESI, thermal analysis (TGA, DSC), FT-IR and XRD measurements. Crystals were obtained in DMSO. ¹H-NMR and MS-ESI titrations were performed with Boc(β ala) $_2$ N $_2$ H $_3$ and AgNO $_3$ in DMSO d $_6$ or D $_2$ O. AgNP formation with Boc(β ala) $_2$ N $_2$ H $_3$ and AgNO $_3$ were recorded overnight by UV-Vis spectroscopy and analysed with TEM, MS-ESI and FT-IR. Different temperature conditions were hereby investigated. Antimicrobial tests were performed with *E.coli* (ATCC 25922) and Boc(β ala) $_2$ N $_2$ H $_3$. The OD $_{600}$ was taken over a time period of 24 h at 37 °C. **RESULTS:** Figure 1a represents the time-resolved formation of AgNP at pH 7 and 60 °C, while 1b depicts the obtained AgNPs. Figure 1c shows the crystal structure of Boc(β ala) $_2$ N $_2$ H $_3$. A first trial of silver complexation with Boc(β ala) $_2$ N $_2$ H $_3$ by NMR titration gave a high-field shift for the hydrazine end group of 1.026 (NH $_2$) and 0.591 ppm (NH). Antimicrobial tests revealed an OD $_{600}$ of 0.7 for just *E.coli*, 0.68 for *E.coli* with 1 mg/ml Boc(β ala) $_2$ N $_2$ H $_3$, and 0.55 for *E.coli* with 2 mg/ml Boc(β ala) $_2$ N $_2$ H $_3$ after 24 h of growth. **DISCUSSION & CONCLUSIONS:** The peptide crystallizes in the orthorhombic space group $P2_12_12_1$. The packing reveals three H-bonds of 2.03–2.13 Å, which connect the peptide molecules into 1D-ribbons, which are themselves connected in an anti-parallel fashion via H-bonds between the hydrazine groups to form dimers of ribbons. These dimers of ribbons are arranged in zigzag into layers, which are themselves assembled through short interactions into a 3D structure with a mean distance of ~ 2.3 Å. First tests showed that Boc(β ala) $_2$ N $_2$ H $_3$ is capable to form AgNPs at 40, 50 and 60 °C but not at RT. For 60 °C the highest absorbance was observed meaning that the reaction is temperature dependent. AgNP formation occurred over several hours, reaching in case of 60 °C sizes of 30 to 90 nm. The NMR titration showed that the interaction with silver takes place mainly at the hydrazine end group. The antimicrobial tests indicated a slight decrease in bacterial growth for 2 mg/ml of Boc(β ala) $_2$ N $_2$ H $_3$.



Figure 1: a) UV-VIS of AgNP formation with 1.25 mM Boc(β ala) $_2$ N $_2$ H $_3$ and 1.25 mM AgNO $_3$ at pH 7 and 60 °C; b) TEM of 30-90 nm big AgNP formed, scale 2 μ m; c) Crystal structure of Boc(β ala) $_2$ N $_2$ H $_3$, R = 3.61 %

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