$Boc(\beta ala)_2N_2H_3$ and its interaction with silver

A. Holzheu[1], A. Crochet[1], K. M. Fromm[1]*

[1]University 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 agent[2]. Therefore, we study the antimicrobial effects of a dipeptide, $Boc(\beta ala)_2N_2H_3$, 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 $Boc(\beta ala)_2N_2H_3$ dipeptide, a standard liquid phase synthesis was used[3]. The dipeptide was characterized by ^{1}H- and ^{13}C-NMR, MS-ESI, thermal analysis (TGA, DSC), FT-IR and XRD measurements. Crystals were obtained in DMSO. ^{1}H-NMR and MS-ESI titrations were performed with $Boc(\beta ala)_2N_2H_3$ and $AgNO_3$ in DMSO d^6 or D_2O. AgNP formation with $Boc(\beta ala)_2N_2H_3$ and $AgNO_3$ were recorded overnight by UV-Vis spectroscopy and analysed with TEM, MS-ESI and FT-IR. Different temperature conditions where hereby investigated. Antimicrobial tests were performed with *E.coli* (ATCC 25922) and $Boc(\beta ala)_2N_2H_3$. 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 $Boc(\beta ala)_2N_2H_3$. A first trial of silver complexation with $Boc(\beta ala)_2N_2H_3$ 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 $Boc(\beta ala)_2N_2H_3$, and 0.55 for *E.coli* with 2 mg/ml $Boc(\beta ala)_2N_2H_3$ 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 $Boc(\beta ala)_2N_2H_3$ 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 $Boc(\beta ala)_2N_2H_3$.

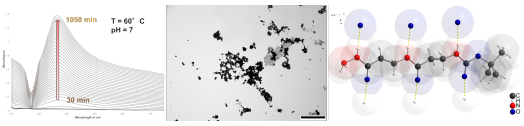

Figure 1: a) UV-VIS of AgNP formation with 1.25 mM $Boc(\beta ala)_2N_2H_3$ and 1.25 mM $AgNO_3$ at pH 7 and 60 °C; b) TEM of 30-90 nm big AgNP formed, scale 2 µm; c) Crystal structure of $Boc(\beta ala)_2N_2H_3$, R = 3.61 %

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