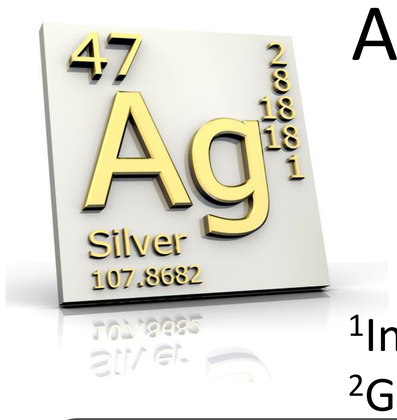
Antibiotic-metal constructs to combat Gram-positive and Gram-negative bacteria resistance



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Introduction

The pathogenic microorganisms cause significant concerns in hospitals and food storage all over the world [1]. Currently antibiotics such as vancomycin, colistin and gentamicin are used to treat bacterial infections; however, there has been an alarming rise in the numbers of multi-drug resistant (MDR) bacteria that are resistant to most antibiotics. One potential solution is to complex antibiotics with metal ions as antimicrobial agents such as copper, zinc and silver to overcome the MDR and improve the antimicrobial effectiveness [2].

With higher concentration of Vancomycin, its monomers tends to bind to each other and form dimers with the sugar region to be back to back. And several vancomycin molecules can bind to each other and form tetramers or hexamers when the concentration is increased. Based on the NMR results obtained in Cooper group we have assumed that at lower concentration vancomycin is mainly in a monomer form which can provide more binding sites for silver ions then its dimer, tetramer, or hexamer. Vancomycin can form stable complexes with copper at the same site as ligand binds [3].

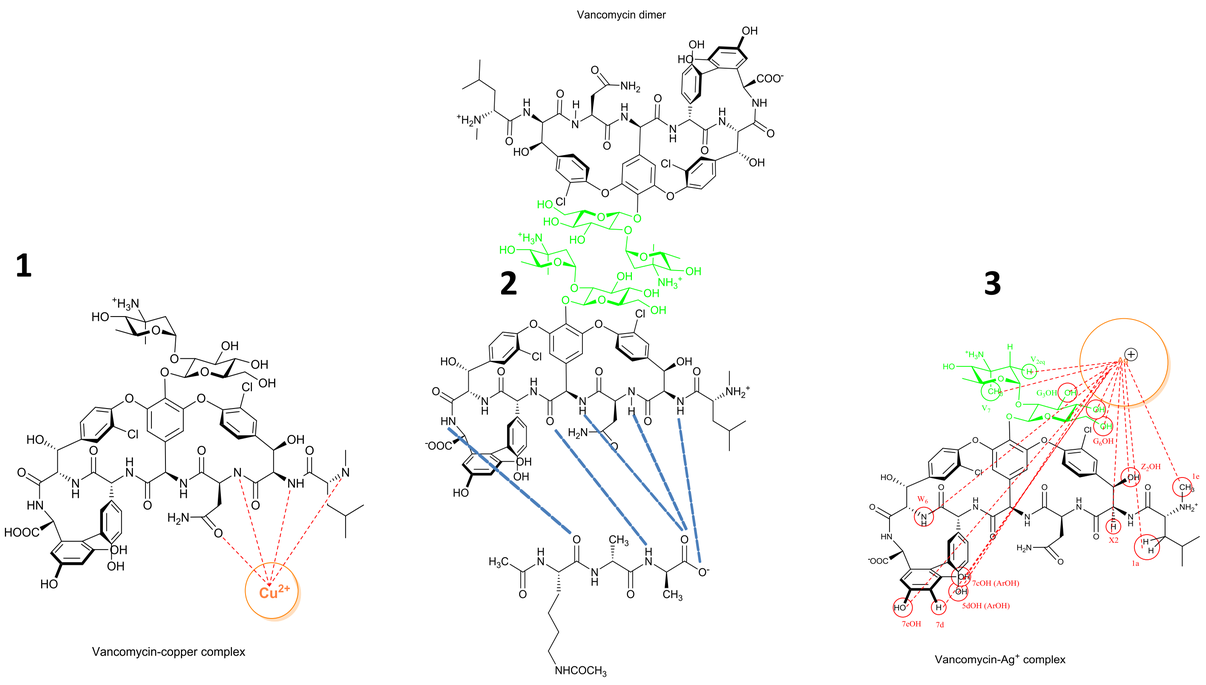

Fig. 1: **1**. Binding sites between Cu(II) and vancomycin; **2**. Binding sites between dimer and ligand; **3**. Binding sites between Ag(I) and vancomycin.

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) is an analytical technique to detect trace metals in different environmental samples. It is an emission spectroscopy which applies the inductively coupled plasma to stimulate atoms and ions [4].

Table 1: ICP-OES measurements.

Compounds	Molar Weight of metal [g/mol]	Molar weight of salts/complexes [g/mol]	Expected percentage %	Determined Percentage %	Purity %
$ZnCl_2$	65.39	136.30	47.98	37.73	78.64
$ZnAc_2$	65.39	219.51	29.79	29.02	97.43
$CuCl_2.2H_2O$	63.55	170.48	37.28	37.15	99.66
$AgNO_3$	107.87	169.87	63.50	63.75	100.4
Ag_2SO_4	107.87	311.80	69.19	61.11	88.32
AgAc (Nathan)	107.87	166.91	64.63	57.19	88.49
AgAc (IMB)	107.87	166.91	64.63	69.08	106.90
Vancomycin+AgAc	107.87	1652.62	6.53	7.02	107.65
Vancomycin+$AgNO_3$	107.87	1655.58	6.52	6.54	100.33

The concentrations of silver salts were in the expected range, except sulfate and old acetate form. $CuCl_2$ showed high purity with over 99% while the amount of Zn(II) in $ZnCl_2$ was much lower because of high hygroscopic nature. No contamination of metal ions (zinc, copper, magnesium, silver, cadmium, palladium, aluminum or iron) were found in antibiotics and complexes.

Reference

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Metal ion complexes by ITC

Isothermal titration calorimetry (ITC) is a calorimetrical technique used to investigate the thermodynamic parameters of interactions in solution. It is most often used to determine the binding affinity of small molecules to larger macromolecules [5]. ITC can determine the thermodynamics and stoichiometry for complexes formed between metal ions and antibiotics.

Table 2: Complexes of metal ions with antibiotics

System	K [10^5 M-1]	ΔG [10^{-3} cal/mol]	ΔH [10^{-3} cal/mol]	ΔS [cal/mol/K]	N
5 mM $CuCl_2$ to 0.75 mM vancomycin pH = 7.5	1.03±0.12	-6.82±0.07	-0.21±0.04	-22.2±0.10	1.06±0.01
5 mM $CuCl_2$ to 0.5 mM vancomycin pH = 7.5	0.735±0.13	-6.63	-0.25±0.00	21.4	1.28±0.02
5 mM $CuCl_2$ to 0.4 mM vancomycin pH = 7.5	1.12±0.10	-6.68±0.16	-0.50±0.04	20.7±0.60	1.32±0.10
5 mM $CuCl_2$ to 0.3 mM vancomycin pH = 7.5	0.84±0.07	-6.71±0.05	-0.48±0.01	20.9±0.2	1.33±0.04
5 mM $CuCl_2$ to 0.4 mM vancomycin, pH = 5.1	0.86±0.22	-6.77	-0.24±0.0	21.9	1.55±0.03
5 mM $CuCl_2$ to 0.3 mM vancomycin, pH = 5.1	1.03±0.29	-6.83	-0.15±0.0	22.4	1.91±0.04
2.5 mM $AgNO_3$ to 0.15 mM vancomycin pH = 7.5	2.96±0.05	-7.46±0.01	-8.18±0.06	-2.40±0.15	1.41±0.01
2.5 mM $AgNO_3$ to 0.1 mM vancomycin pH = 7.5	2.69±0.16	-7.41±0.04	-8.46±0.14	-3.54±0.61	1.48±0.09
2.5 mM $AgNO_3$ to 0.05 mM vancomycin pH = 7.5	2.18±0.26	-7.27±0.07	-5.22±0.19	6.88±0.89	2.41±0.01
1.5 mM $AgNO_3$ to 0.025 mM vancomycin pH = 7.5	2.62±0.14	-7.38±0.03	-1.90±0.06	18.4±0.3	4.97±0.00
2.5 mM $AgNO_3$ to 0.15 mM vancomycin pH = 5.1	6.60±0.35	-7.94±0.02	-14.63±0.18	22.45±0.65	1.20±0.02
2 mM $AgNO_3$ to 0.1 mM vancomycin pH = 5.1	4.00±0.05	-7.64±0.01	-9.14±0.09	-5.03±0.32	2.12±0.04
1.5 mM $AgNO_3$ to 0.05 mM vancomycin pH = 5.1	3.53±0.02	-7.57±0.00	-7.86±0.02	-0.96±0.08	2.81±0.03
0.75 mM $AgNO_3$ to 0.025 mM vancomycin pH = 5.1	7.78±1.04	-8.03±0.08	-3.96±0.05	13.65±0.45	3.43±0.12
1.5 mM Ag_2SO_4 to 0.15 mM vancomycin pH = 7.5	1.73±0.00	-6.95±0.19	-5.35±0.12	-6.02±0.40	2.46±0.04
0.75 mM Ag_2SO_4 to 0.05 mM vancomycin pH = 7.5	4.39±0.21	-7.68±0.03	-1.01±0.13	22.4±0.90	3.49±0.18
1.5 mM Ag_2SO_4 to 0.15 mM vancomycin pH = 5.1	4.01±0.01	-7.65±0.00	-8.55±0.04	-3.07±0.14	2.08±0.03
0.75 mM Ag_2SO_4 to 0.05 mM vancomycin pH = 5.1	4.15±0.02	-7.66±0.01	-5.85±0.02	6.07±0.11	2.39±0.01
2.5 mM AgAc to 0.15 mM vancomycin pH = 7.5	2.91±0.30	-7.45±0.06	-9.44±0.24	-6.67±1.01	1.40±0.03
1 mM AgAc to 0.05 mM vancomycin pH = 7.5	10.26±1.94	-8.16±0.02	-0.90±0.20	24.30±0.80	2.62±0.11
2.5 mM AgAc to 0.15 mM vancomycin pH = 5.1	8.28±0.57	-8.06±0.04	-13.47±0.09	-18.2±0.04	1.42±0.07
1 mM AgAc to 0.05 mM vancomycin pH = 5.1	6.70±0.64	-7.92±0.03	-4.60±0.15	11.20±0.70	2.43±0.02
1 mM AgAc to 0.025 mM vancomycin pH = 5.1	5.84±1.55	-7.83±0.15	-1.40±0.02	21.60±0.60	4.14±0.12
2.5 mM AgAc to 0.15 mM gentamicin in H_2O	18.5±1.9	-8.55±0.07	-14.75±0.11	-20.8±0.6	2.02±0.02

Conclusion

- The interactions between zinc salts and vancomycin are not strong and no complex formation was observed.
- The binding between copper ion and vancomycin is stable and the affinity is much stronger than for zinc.
- Strong heat response and great energy changes observed in the ITC demonstrate a strong affinity between silver ion and vancomycin.
- The interactions between silver salts and colistin are not strong and no complex formation was observed.
- The binding between silver ion and gentamicin was observed only when silver was in the acetate form.
- Complexation of vancomycin with silver ions could be a promising new way to increase its antimicrobial efficacy and help to overcome multidrug resistance.