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Biofilm inhibition by a new Mn(II) complex with sulfamethoxazole: Synthesis, spectroscopic characterization and crystal structure

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ABSTRACT

The reaction in alkaline aqueous solution between sulfamethoxazole (SMX) and manganese (II) chloride produces colorless crystals with formula $[Mn(H_2O)_6]_{0.5}[Mn(SMX)_3]$, which was characterized by UV–Vis, IR and Raman spectroscopy and thermal analysis. The crystal structure of $[Mn(H_2O)_6]_{0.5}[Mn(SMX)_3]$ complex has been solved by X-ray diffraction methods. It crystallizes in the cubic *Pa*-3 space group with $a = 19.5476(1)$ Å, and $Z = 8$ molecules per unit cell. $[Mn(SMX)_3]^-$ complex is at a crystallographic special position of C_3 symmetry with the Mn(II) ion *cis*-coordinated to three equivalent sulfamethoxazole molecules acting as bidentate ligands in a propeller-like conformation. $[Mn(H_2O)_6]^{2+}$ hydrate ion is at crystal special position of S_6 symmetry with the metal in an almost perfect octahedral coordination with six water molecules. At 100 μg/mL $Mn(H_2O)_6]_{0.5}[Mn(SMX)_3]$ and SMX, inhibited the *Staphylococcus aureus* biofilm formation by 92% and 54%, respectively. However, at the same concentration $Mn(H_2O)_6]_{0.5}[Mn(SMX)_3]$ and SMX inhibited the planktonic bacterial growth by 52% and 81%, respectively. The Mn(II) complex inhibited the biofilm formation in values higher than 35% at the concentration 0.5 μg/mL. These results suggest that the metal complex $[Mn(H_2O)_6]_{0.5}[Mn(SMX)_3]$ is a good candidate for the development of new antimicrobial agent acting in part as bactericidal but mainly as antipathogenic agent.

1. Introduction

Sulfonamides were the first effective chemoterapeutic agents employed systematically for the prevention and cure of bacterial infections in humans. They are the drugs of choice for the treatment of chancroid, nocardiosis and acute urinary tract infections caused by several microorganisms including *Escherichia coli* and *Proteus mirabilis*. These substances can be used combined with other drugs in the treatment of otitis, meningitis, toxoplasmosis, recurrent and chronic urinary tract infections and in diarrhea, among other diseases [1–3]. Sulfonamides were also used in a variety of applications including antitumor agents [4], diuretics [5], carbonic anhydrase inhibitors [6], hypoglycaemic agents [7], and thyroid and protease inhibitors [8,9]. Recently, it was reported the presence of pathogens with sulfonamide-resistance genes in drinking water [10].

However, all the above-mentioned studies were carried out considering the bacteria as unicellular life forms. Direct observation of a wide variety of natural habitats has established that the 99% of microbes persist attached to surfaces within a structured biofilm ecosystem and not as free-floating organisms [11]. Today it is well-known the prevalence of biofilms in chronic infections and on medical implants. Bacteria that form biofilms can withstand host immune responses and are much more resistant to antibiotics than their counterpart planktonic bacteria. This effect is due to protective features of the film such as impedance against diffusion and favorable environment within the film [12].

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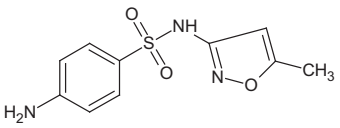
Therefore, infections with biofilm forming bacteria are persistent and difficult to treat with antibiotics. Bacteria in biofilms survive exposure to concentrations of antibiotics 1000-fold greater than the one that are lethal when the free cells are in suspension [12]. For this reason, the development of anti-infective compounds, which are active not only against planktonic microorganisms but mainly also against biofilms represents an imperative goal [13].

The chemistry of metal complexes with heterocyclic compounds containing nitrogen, sulfur, and/or oxygen as ligand atoms has attracted increasing attention. It is well-known that metal-based therapeutics for treatment of many ailments have gained much attention during the past decade. The ability of metal ions to bind *in vivo* with proteins and peptides is an important feature of metal-based drugs. Simple and N-substituted sulfonamides have attracted much attention in this context. The development of new metal complexes with sulfonamides is an important field of research, considering that one can combine the specific antibacterial activities of the sulfonamides and the multi-targeting antimicrobial activities of the metal ions. In many cases, the metal complex exhibits a better activity than the free ligand at the same experimental conditions [14]. In particular, silver sulfonamides compounds have proved to be effective topical antimicrobial agents, especially Ag-Sulfadiazine (Ag-SDZ) used in burn therapy [15]. Ag-SDZ has shown to be insoluble in water and in other common organic solvents, which limits its application in medicine.

Sulfamethoxazole (SMX) was part of the second generation of sulfonamides (see Scheme 1) and it is used in a synergistic combination with trimethropim. SMX is the drug most used to treat infections produced by *Pneumocystis pneumonia*, which is a form of pneumonia caused by a yeast-like fungus that affects patients with HIV [16]. There are also some metal complexes of sulfamethoxazole reported in the literature [17–26]. Two Cd(II) complexes of sulfamethoxazole were obtained and their crystal structures were reported [19,20]. In $[Cd(SMX)_2(CH_3OH)_2]$, the Cd(II) centers are linked through sulfamethoxazolate anions which alternate in their coordination with the isoxazolic N-atoms and the aromatic amino groups [19]. A similar structure was obtained for $[Cd(SMX)_2(L)_2]$ complexes (L = Dimethylformamide, dimethyl sulfoxide and pyridine) [20]. Mondelli et al. have reported the synthesis and structural characterization of $[Co(SMX)_2(H_2O)_2]\cdot H_2O$ complex, where Co(II) is in a slightly tetragonally distorted octahedron where the SMX molecules act as a head-to-tail bridges between two Co atoms forming polymeric chains [23]. Marques et al. have synthesized Au(I) and Ag(I) complexes with SMX as ligand. Both complexes present a linear geometry and the SMX bind to Au and Ag through the N of the sulfonamide group [22]. The antibacterial activities of both complexes were determined and the gold complex showed greater activity against *E. coli* and *Staphylococcus aureus* than the silver one [22]. However, there are not information about the effect of sulfamethoxazole complex against bacterial biofilm.

In the present contribution we report the synthesis and spectroscopic characterization of a new Mn(II) complex with sulfamethoxazole as ligand with formula $[Mn(H_2O)_6]_{0.5}[Mn(SMX)_3]$. The crystal structure was determined by single-crystal X-ray diffraction methods. To the best of our knowledge, no previous study on the anti-biofilm activity of sulfamethoxazole complex against *S. aureus*.



Scheme 1. Structure of sulfamethoxazole.

2. Experimental

2.1. Synthesis of $[Mn(H_2O)_6]_{0.5}[Mn(SMX)_3]$ complex

The Mn(II) complex with sulfamethoxazole was synthesized by mixing together aqueous solutions of the appropriate $MnCl_2\cdot 4H_2O$ (1 mmol) and sodium sulfamethoxazole (2 mmol) under continuous stirring at room temperature (RT). The precipitate formed was separated by filtration and the slow evaporation of the remaining solution gave suitable crystals for structural X-ray diffraction. The complex was soluble and stable in water, dimethylsulfoxide (DMSO) and dimethylformamide (DMF).

2.2. Crystallographic data and structure determination

The X-ray measurements were performed on an Oxford Xcalibur, Eos, Gemini CCD diffractometer with graphite-monochromated CuKα (λ = 1.54184 Å) radiation. X-ray diffraction intensities were collected (ω scans with θ and κ-offsets), integrated and scaled with CrysAlisPro [27] suite of programs. The unit cell parameters were obtained by least-squares refinement (based on the angular settings for all collected reflections with intensities larger than seven times the standard deviation of measurement errors) using CrysAlisPro. Data were corrected empirically for absorption employing the multi-scan method implemented in CrysAlisPro. The structure was solved by direct methods with SHELXS-97 program of the SHELX package [28] and the corresponding molecular model developed by alternated cycles of Fourier methods and full-matrix least-squares refinement with the program SHELXL-97 of the same package. All H-atoms were located in a Fourier difference map phased on the heavier atoms and refined at their found positions with isotropic displacement parameters. The methyl group converged to a staggered conformation. Crystal data, data collection procedure, structure determination methods and refinement results are summarized in Table 1. Crystallographic structural data have been deposited at the Cambridge Crystallographic Data Centre (CCDC). Any request to the CCDC for this material should quote the full literature citation and the reference number CCDC 1057060.

2.3. Physical measurements

The FTIR absorption spectrum of the solid state compound was recorded from KBr pellets in the 4000–400 cm^{-1} frequency range with a Perkin–Elmer GX1 Fourier Transform infrared instrument. The corresponding Raman dispersive spectrum was measured in the 3500–50 cm^{-1} interval with a Thermoscientific DXR Raman microscope. The Raman data were collected (at 5 cm^{-1} spectral resolution) using a diode-pump solid state laser of 532 nm wavelength, a con-focal aperture of 25 μm pinhole and 10x objective. The sample was placed on gold-coated sample slides. To achieve a sufficient signal to noise ratio, 30 spectral scans of 2 s each were accumulated during the measurements with the laser power maintained at 10 mW. UV–Vis measurements were recorded using quartz cells (10 mm optical path length) on a Beckman/DU 7500 spectrophotometer. For this purpose, a solution of 10^{-4} mol/L of the complex in DMSO was prepared. The spectrum was recorded between 800 and 200 nm. Calorimetric measurements were performed using a differential scanning calorimeter Perkin Elmer Pyris DSC 6. The experiments were carried out using 3.980 mg of powdered sample sealed in aluminum pans with a mechanical crimp. Temperature and heat flow calibrations were made with standard samples of indium by using its melting transition. Enthalpy changes associated with the dehydration of the sample in study (ΔH) was directly obtained from the DSC data by