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Copper(II) complexes with cyanoguanidine and o-phenanthroline: Theoretical studies, *in vitro* antimicrobial activity and alkaline phosphatase inhibitory effect

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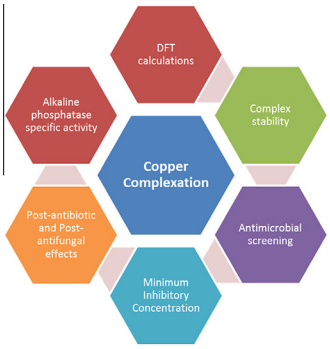
HIGHLIGHTS

- The calculated geometrical parameters are in agreement with the experimental values.
- The complexation increased: The antibacterial activity against *E. faecalis*. The post antibiotic and antifungal effects against *E. coli* and all fungal strains.
- The alkaline phosphatase inhibitor activities of the metal and cyanoguanidine.

GRAPHICAL ABSTRACT



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ABSTRACT

Calculations based on density functional methods are carried out for two Cu(II) complexes with cyanoguanidine (cnge) and o-phenanthroline (o-phen): $[Cu(o\text{-}phen)_2(cnge)](NO_3)_2{\cdot}2H_2O$ (1) and $[Cu(o\text{-}phen)(cnge)(H_2O)(NO_3)_2]$ (2). The calculated geometrical parameters are in agreement with the experimental values. The results of Atoms in Molecules (AIM) topological analysis of the electron density indicate that the Cu–N(phen) bonds in complex (1) have lower electron density, suggesting that those bonds are stronger in complex (2). Moreover, the ionic character of the Cu–N bond in the complex (1) is slightly stronger than that in the complex (2) and this situation would explain the fact that only complex (2) was stable in water solution. For this reason, the antimicrobial and enzymatic assays were performed using complex (2). It is well known that the increased use of antibiotics has resulted in the development of resistant bacterial and fungal strains. In this context, the study of novel antimicrobial agents has an enormous importance and metal complexes represent an interesting alternative for the treatment of infectious diseases. The aim of this work is to prove the modification of some biological properties like antimicrobial activity or alkaline phosphatase inhibitory activity upon copper complexation.

The antimicrobial profile of the metal, the ligands and complex (2) was studied against several bacterial and fungal strains by different microbiological methods. The values of MIC indicate that the complexation increases the antibacterial activity against *Enterococcus faecalis*, but decreases this activity against *Pseudomonas aeruginosa*. The complex (2) exhibited longer PAEs/PAFEs than copper and o-phen against *Escherichia coli* and all fungal strains, and longer PAEs/PAFEs than some antibiotics or antifungal agents against *E. coli*, *Staphylococcus aureus* and *Candida albicans*. Complexation also improves the alkaline

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phosphatase inhibitory effect of copper and cnge. Therefore, the interaction of copper(II) with N-containing ligands may provide a promising strategy for the development of novel drugs with enhanced antimicrobial activity or alkaline phosphatase inhibitory activity.

1. Introduction

Cyanoguanidine (cnge) has been recently recognized as nitrogenase substrate. The dimer functions as a dehydration coupling agent that links glucose and adenosine to phosphoric acid and then forms glucose-6-phosphate and adenosine-5′-phosphate, respectively [1]. Despite its biological importance, cnge also has commercial applications as intermediate in the formation of pharmaceuticals, pesticides, fungicides, and various polymers. Some transition metal ions (cf. copper, platinum and nickel) are able to catalyze the addition of alcohols to the nitrile group in cnge, forming n-alkylguanylureas that coordinates the metal ions [2–5].

In our previous paper [6], we report the molecular structure, bioavailability and bioactivity of two Cu(II) complexes with cnge and o-phenanthroline (o-phen), $[Cu(o\text{-phen})_2(cnge)](NO_3)_2{\cdot}2H_2O$ (1) and $[Cu(o\text{-phen})(cnge)(H_2O)(NO_3)_2]$ (2). The X-ray structure of the complex (1) reveals that the copper(II) ion is in a trigonal bipyramidal environment, coordinated to two o-phenanthroline groups acting as bidentate ligands through their N-atoms and to the cyanide N-atom of a cyanoguanidine molecule that enters coordination with a bent Cu–N–N angle. In the complex (2), the metal ion is at the center of a strongly elongated octahedral environment, equatorially coordinated to an o-phenanthroline ligand through its N-atoms, to the cyanide N-atom of a cyanoguanidine molecule that enters coordination radially and to a water molecule along the oxygen electron lone pair. The distorted octahedral coordination around copper is completed with two nitrate ions at the axial positions. As part of a continuing investigation on these complexes, in this paper we report calculations based on density functional theory (DFT), together with AIM analysis for the interactions between metal and the ligands. A charge density analysis will provide significant insight into the properties of the molecule as the total electron density distribution defines all the molecular properties in the ground state [7].

It is well known that copper is an essential trace element for many biological functions; however, in certain amounts copper can have toxic effects against microorganisms [8,9]. Besides, the antimicrobial action of o-phen has been demonstrated on several species of bacteria and fungus in some previous reports [10,11]. Moreover, the antimicrobial profile of some compounds can be modified upon complexation with copper ion and some copper complexes with antimicrobial activity have been studied [8,12–14]. Additionally, the increased use of antibiotics has resulted in the development of resistant strains. In this context, it is interesting to note the enormous importance of developing novel antimicrobial drugs for the treatment of infectious diseases [12,15]. In view of this, we have investigated the antimicrobial activity of copper, cnge, o-phen and complex (2) by agar diffusion and agar dilution methods for Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungus (*Candida parapsilosis* ATCC 22019, *Candida tropicalis* and *Candida albicans* of clinical isolates).

On the other hand, persistent suppression of bacterial growth following short antibiotic exposure has been well documented with a variety of microorganisms; this phenomenon is known as post-antibiotic effect (PAE) [16]. The PAE is the lag phase or recovery period of bacterial growth after brief exposure to an antibiotic. The PAE is a pharmacodynamic parameter and the presence of this effect may be an important consideration in designing antibiotic dosage regimens [17,18]. Determination of the PAE is recommended in pre-clinical evaluation of all new antimicrobial agents because it is a factor that influences optimal antimicrobial dosing intervals [19]. Also, the post-antifungal effect (PAFE) was previously defined as the time in which the antifungal agent was capable of causing growth suppression of the organism following limited exposure to the tested agents [20]. In the present study, the PAE for three strains of bacteria and the PAFE for three strains of fungi were studied. The PAEs/PAFEs were induced by exposure to twice the MIC ($2 \times$ MIC) of all compounds (metal, ligand and complex 2) for 1 h at 37 °C. The point at which microbial growth occurred was determined using a spectrophotometric method.

Alkaline phosphatase (ALP), as a group of ubiquitous enzymes, catalyzes the non-specific hydrolysis of phosphate monoester. ALP activity in serum is usually correlated with bone and liver diseases in vivo and is a marker of osteoblastic differentiation. The analysis of serum ALP has been extensively applied in routine clinic diagnosis [21]. It is demonstrated that some copper(II) complexes inhibits ALP activity [22,23]. The effect of the complex (2) on ALP activity has also been tested in order to determine a possible mechanism of the antimicrobial action of the compounds, by means of enzymatic inhibition.

2. Materials and methods

2.1. Reagents and instrumentation

All chemicals were of analytical grade. Copper(II) chloride dihydrate ($CuCl_2{\cdot}2H_2O$) was obtained from Merck, para-nitrophenyl phosphate (p-NPP), bovine intestinal ALP, solvents and all the other analytical grade chemicals used were purchased from Sigma. The growth media (Mueller Hinton Broth and Mueller Hinton Agar) and blank sterile discs were purchased from Brithania and Bioartis, respectively. $[Cu(o\text{-phen})(cnge)(H_2O)(NO_3)_2]$ complex was prepared according to the preparative technique described in our previous report [6]. Electronic absorption spectra were recorded on a spectrophotometer Agilent Technologies (Cary 60 UV–Vis).

2.2. Computational methodology

Calculations were performed using the GAUSSIAN 03 program package [24]. Molecular structures of ligands and metallic complexes were fully optimized at various levels. Geometry optimization procedures were started from the experimental crystallographic data employing the HF level with the 3-21G* basis set, and later, the effect of electron correlation on the molecular geometry was taken into account by using Becke's three-parameter hybrid, and the gradient corrected Lee–Yang–Parr correlational functional (B3LYP) [25] employing 6-31G(d,p) and 6-31+G(d,p) basis set.

Orthogonal rotations are commonly used for comparing macromolecular structures, and the root mean square deviation (RMSD) is a natural metric for quantitating the similarity of two optimally rotated structures [26]. To test the validity of quantum chemical calculations in reproducing the experimental structure of metal complexes RMSD between the coordinates of both macromolecules were calculated using Qmol package [27].