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DFT vibrational assignments, *in vitro* antifungal activity, genotoxic and acute toxicity determinations of the $[Zn(phen)_2(cnge)(H_2O)](NO_3)_2 \cdot H_2O$ complex

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ABSTRACT

Calculations based on density functional methods were carried out for the $[Zn(phen)_2(cnge)(H_2O)](NO_3)_2 \cdot H_2O$ complex taking into account the presence of two different conformers for the cyanoguanidine ligand. The calculated geometrical parameters and the vibrational IR and Raman spectra were in agreement with the experimental data. On the other hand, the activities of the complex, the ligands and the metal against fungal strains have been measured. The complexation increased the antifungal activity of the metal and the ligand cyanoguanidine, and slightly decreased the antifungal activity of the ligand 1,10-phenanthroline against *Candida albicans*, *C. albicans* ATCC 10231 and *Candida krusei* (not against the others strains of Candida). The ligand 1,10-phenanthroline and the zinc complex showed in some cases higher activity than the common antifungal drug fluconazole. The complexation also increased the post-antifungal effect in the tested strains, except for *Candida parapsilosis*, even with a better efficiency than those of some conventional antifungal agents. Antifungal studies were coupled with safety evaluations using the *Artemia salina* and the Ames tests. The zinc complex behaved as a non-mutagenic and non-toxic compound at the tested concentrations. Moreover, the zinc complex could be safer than the ligand when used as an antifungal agent. Therefore, the interaction of zinc(II) with N-containing ligands may provide a promising strategy for the development of novel and more secure drugs with antifungal activity.

1. Introduction

Cyanoguanidine (cnge) has been widely employed as a ligand of the transition metals in the synthesis of coordination compounds [1,2]. Its solid state structure has raised several theoretical and experimental studies suggesting the coexistence of two tautomeric forms: cyanoimine and cyanoamine [3] (Scheme 1).

Despite its biological importance, cnge has also commercial applications as an intermediate in the formation of pharmaceuticals, pesticides, fungicides, and various polymers. Some transition metal ions (i.e. 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 [4–7]. On the other hand, 1,10-phenanthroline (phen) can act as a strong field bidentate ligand that forms very stable chelates with many transition metals of the first row [8]. This molecule has antibacterial, antifungal and antiviral properties [9]. Besides, it is well known that zinc is an essential metal, widely distributed in cells, and is the most abundant intracellular trace element. Catalytic, structural, and regulatory are the main biological roles played by the biometal [10].

The development of resistant strains is increasing as a result of excessive antibiotic use. In this context, it is interesting to note the enormous importance of developing novel antimicrobial drugs for the treatment of infectious diseases [11,12]. The antimicrobial action of phen has been demonstrated on several bacterial and fungal strains [13,14]. Zinc is an essential element involved in many vital cellular reactions at its low endogenous concentrations. When the concentrations of Zn(II) ion became higher than the optimal range, the ions can enter inside the microbial cells producing cytotoxic effects on the prokaryotic cells. The antimicrobial activity of Zn(II) is

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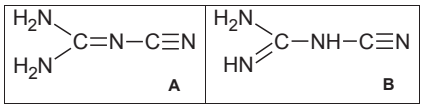

Scheme 1. Structures of cyanoimine (A) and cyanoamine (B).

essentially dependent on the microbial strain [15]. In this way, Zn(II) might be able to display its antimicrobial activity acting as either antibacterial or antifungal agent. Moreover, the antifungal profile of some compounds can be modified upon complexation with zinc ion [13,16–22]. These Zn(II) complexes exerts antifungal action against some Candida and others fungal strains, whereas other complexes like $[Zn(Hz_2DAP\text{-}2H)]\cdot H_2O$ (with Hz2DAP: bis(phthalazine-1-hydrazone)-2,6-diacetylpyridine) promotes the growth of *Candida albicans* [23].

In our previous paper [24], we reported the structural, spectral and potentiometric characterization, and the antibacterial activity of the $[Zn(phen)_2(cnge)(H_2O)](NO_3)_2\cdot H_2O$ complex. The X-ray structure of the complex reveals that the Zinc(II) ion is located in a distorted octahedral environment, coordinated to two nearly planar [*rms* deviation of atoms from the best least-squares plane less than 0.055 Å] bidentate and mutually perpendicular phen [dihedral angle of 88.82(3)°] [Zn–N bond lengths ranging from 2.124(2) Å to 2.193(2) Å]. The remaining two *cis*-positions are occupied by cyanide nitrogen of cnge [d(Zn–N) = 2.092(2) Å] that enters coordination slightly bent [∠ (Zn–N–C) = 161.1(2)°] and a water molecule [d(Zn–Ow) = 2.112(2) Å]. To extend this study, we report herein the calculations of the geometry and the vibrational behavior based on density functional theory (DFT) [25–27] and compared these results with the previous experimental data obtained by X-ray, FT-IR and FT-Raman measurements. We have also investigated the antifungal activity by the agar diffusion and the agar dilution methods and the post-antifungal effect (PAFE) for the Zn(II) ion, the ligands cnge and phen and the zinc complex against seven strains of Candida. The antifungal studies were coupled with the evaluation of the mutagenicity and acute toxicity of all compounds.

2. Experimental

2.1. Materials

All chemicals were of analytical grade. Anhydrous Zinc(II) chloride ($ZnCl_2$) was obtained from Merck, 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 Britannia and Bioartis, respectively. The zinc complex was prepared according to the preparative technique described in our previous report [24]. Electronic absorption spectra were recorded on a spectrophotometer Agilent Technologies (Cary 60 UV–vis).

2.2. Methods

2.2.1. Computational methodology

The X-ray diffraction data was employed as the starting structure to optimize the geometry of the zinc complex using tools from the DFT. Calculations were performed using the GAUSSIAN 09 program package [28]. Molecular structures of ligands and metallic complexes were fully optimized at various levels. Geometry optimization procedures were started from the experimental crystallographic data using the Beck three-parameters hybrid exchange-correlation functional, known as B3LYP [29] employing different basis set for different atoms. Theoretical calculations were carried out simulating water environment using the conducting polarizable continuous model (CPCM) as implemented in the software package [30]. Basis sets of triple-zeta quality with polarized (TZVP) functions for atoms of Zn, C and H, and a TZVP basis set with diffuse functions (TZVPD) for oxygen and nitrogen atoms [31].

IR absorption and Raman spectra of cnge in different physical states (crystalline, solutions and composition with porous glasses) reveals the co-existence of two different structural cnge species: cyanoimine and cyanoamine [3]. Theoretical studies of the cnge isomers confirm that these two forms co-exist at the same time [3,32]. Accordingly, it was suggested that the X-ray diffraction data showing equivalent C–N and C=N bond lengths in the guanidine fragment is a consequence of the superposition of both tautomers that are present simultaneously in cnge samples. Taking into account these findings, the cation complex geometry optimization was carried out considering both cyanoguanidine tautomers individually, 1-cyanoguanidine (cyanoamine) and 2-cyanoguanidine (cyanoimine). The structures of the zinc complexes optimized with cyanoamine and cyanoimine were called (**1**) and (**2**), respectively.

Vibrational spectroscopy is one of the most important and promising tools for the characterization of the structural features of molecules (backbone or functional groups). The combination of theoretical calculations with IR and Raman spectroscopies provide invaluable structural information [33]. Vibrational calculations at the same level of theory were performed to determine the consistency of the minimum in the potential energy surface and to assign the theoretical vibrational spectra.

Orthogonal rotations are commonly used for comparing macromolecular structures, and the root mean square deviation (RMSD) is a natural metric for the quantization of the similarity of two optimally rotated structures [34]. To test the validity of quantum chemical calculations in reproducing the experimental structure of the zinc complex RMSD between the coordinates of both macromolecules were calculated using Qmol package [35].

2.2.2. Stability studies

In order to evaluate that in the *in vitro* studies the effects observed by the dissolved complex are due to the complex itself and not to its dissociation products, stability studies of the zinc complex under the same conditions of antimicrobial and toxicological assays were performed. The dissolution has been carried out in distilled water and in artificial seawater. Stability determinations were followed spectrophotometrically (variation of the electronic absorption spectra with time) at least for 30 min that is the time of manipulation of the solid dissolved before it was employed in the different assays.

2.2.3. Antifungal assays

The antifungal profile of the metal ($ZnCl_2$), the ligands (cnge and phen) and the complex have been studied against seven fungal strains by two different microbiological methods (agar diffusion and agar dilution methods). PAFE measurements were performed by a spectrophotometric method [36].

Control strains for the agar diffusion and agar dilution methods included seven strains of Candida namely: *Candida parapsilosis* ATCC 22019, *C. albicans* ATCC 10231, and clinical isolates of *Candida tropicalis*, *Candida krusei*, *Candida glabrata*, *C. parapsilosis* and *C. albicans*. Mueller Hinton Broth (MHB) or Mueller Hinton Agar (MHA) have been used for the cultivation/assay medium for all strains. The inocula of fungal strains were prepared from 18 h-old broth cultures. A McFarland 0.5 suspension was prepared for each isolate (~10^8 colony forming units (CFU) per mL, CFU mL^{-1}) and employed in all assays [12,37].

Stock solutions were prepared at different concentrations for