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Anti-thyroid and antifungal activities, BSA interaction and acid phosphatase inhibition of methimazole copper(II) complexes

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ABSTRACT

It has been reported that various metal coordination compounds have improved some biological properties. A high activity of acid phosphatase (AcP) is associated to several diseases (osteoporosis, Alzheimer's, prostate cancer, among others) and makes it a target for the development of new potential inhibitors. Anti-thyroid agents have disadvantageous side effects and the scarcity of medicines in this area motivated many researchers to synthesize new ones. Several copper(II) complexes have shown antifungal activities. In this work we presented for a first time the inhibition of AcP and the anti-thyroid activity produced by methimazole–Cu(II) complexes. Cu–Met ($[Cu(MeimzH)_2(H_2O)_2](NO_3)_2{\cdot}H_2O$) produces a weak inhibition action while Cu–Met–phen ($[Cu(MeimzH)_2(phen)(H_2O)_2]Cl_2$) shows a strong inhibition effect (IC_{50} = 300 μM) being more effective than the reported behavior of vanadium complexes. Cu–Met–phen also presented a fairly good anti-thyroid activity with a formation constant value, $K_c = 1.02 \times 10^{10}\ M^{-1}$ being 10^6 times more active than methimazole ($K_c = 4.16 \times 10^4\ M^{-1}$) in opposition to Cu–Met which presented activity ($K_c = 9.54 \times 10^3\ M^{-1}$) but in a lesser extent than that of the free ligand. None of the complexes show antifungal activity except Cu–phen (MIC = 11.71 μg mL^{-1} on *Candida albicans*) which was tested for comparison. Besides, albumin interaction experiments denoted high affinity toward the complexes and the calculated binding constants indicate reversible binding to the protein.

1. Introduction

In recent years, the study of enzyme inhibition mediated by coordination metal compounds has significantly increased as a consequence of the development of the applications of these compounds in medicine. A variety of metal complexes have been studied for this purpose including, complexes of Au(I), Pt(II), V(IV)O [1,2] and Cu(II) [3]. Recently, it has been demonstrated that copper(II) complexes acted as inhibitors of the protein tyrosine phosphatase (PTP) [4] and the alkaline phosphatase (ALP) enzyme [5,6].

On the other hand, purples acid phosphatases (PAP) are metalloenzymes found in animals, plants and fungi. These phosphatases have a binuclear structure with two different metal centers for the catalytic reaction of the hydrolysis of phosphate esters and anhydrides under acidic conditions (one is Fe(III) and the second one could be Fe(II) (in mammals) [7], or Zn(II) or Mn(II) (in plants) [8]). Basically, the structure and the mechanism of action are similar to that of alkaline phosphatase (ALP) and the difference would be the presence of a serine (Ser) amino acid residue [9] whereas in PAP a histidine (His) residue is present. Different biological roles have been proposed for PAP (iron transport during gestation, bone resorption in osteoclasts, catalysis of Fenton's reaction, etc) [10] and its elevated levels in serum are correlated with the progression of osteoporosis (bone metastases), Guacher and Alzheimer's diseases, hyperparathyroidism and prostate cancer, among others [11–13]. The activity of phosphatase acid could be inhibited by certain metal ions such as Hg(II), Cr(VI), Bi(III) [14], Cd (II) [15], some oxo-anions as $HAsO_4^{-2}$, WO_4^{-2} and MoO_4^{-2} [16], some organic compounds [17] but there is not sufficient evidence in the literature

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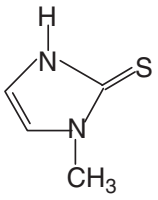

Fig. 1. Chemical structure of methimazole.

concerning to the inhibitory tests performed with coordination compounds and we only found some studies for vanadium compounds [18]. Taking into account that high levels of this enzyme are associated with numerous diseases it turns out to be a good target for the development of new potential inhibitors.

In addition to the possibility of enzymatic inhibition, it is well known that methimazole (Fig. 1) is an anti-thyroid agent which most important effect is the inhibition of the thyroid hormone synthesis by interfering with thyroid peroxidase-mediated iodination of tyrosine residues in thyroglobulin (an important step in the synthesis of thyroxine and triiodothyronine). Disadvantageous side effects related with the drugs have been detected during the past years [19] inspiring researchers to synthesize new anti-thyroid agents with lesser side effects. However, no studies were found concerning the anti-thyroid effects of metal complexes. In consequence we decide to simulate *in vitro* the action of the present copper(II)-methimazole complexes as anti-thyroid drugs [20,21].

In recent years coordination complexes have been studied because of their potential biological applications. It has been revealed in many cases, an improvement of the activity. For this reason, we presented in this work, for a first time, the inhibition acid phosphatase studies and anti-tyroid activity of methimazole copper complexes. We also determined its antifungal and the albumin transport abilities. As it is known, serum albumin is the major transporter protein for unesterified fatty acids and also is capable to bind an extraordinarily diverse range of metabolites, drugs, dyes and organic compounds. Because the metabolism, distribution and efficacy of many drugs in the body are associated with their affinities towards serum albumin, the analysis of compounds with respect to albumin binding ability becomes a relevant item to analyze.

2. Materials and methods

2.1. Reagents and instrumentation

All chemicals were of analytical grade. Bovine Serum Albumin BSA (A-6003, essentially fatty acid-free) and acid phosphatase AcP (from potato, 0.8 U/mg, Deisenhofen, product number P-3752) were obtained from Sigma Chemical Company (St. Louis, MO) and used as supplied. Copper(II) nitrate trihydrate and Copper(II) chloride dihydrate were obtained from Merck. Methimazole, 1,10-phenanthroline monohydrate, para-nitrophenyl phosphate (p-NPP) and all the other analytical grade chemicals used were purchased from Sigma. Methimazol (MeimzH)–copper(II) complexes ($[Cu(MeimzH)_2(H_2O)_2](NO_3)_2{\cdot}H_2O$ (Cu–Met) and $[Cu(MeimzH)_2(phen)(H_2O)_2]Cl_2$ (Cu–Met–phen) and $[Cu(phen)_2Cl]Cl{\cdot}H_2O$ (Cu–phen) were prepared and purified according to published procedures [5,6].

2.2. Acid phosphatase inhibition test

Acid phosphatase inhibition test was performed according to Blum and Schwedt procedures [14]. Acetate buffer was prepared by dissolving a volume of 5.72 mL of concentrated acetic acid in distilled water (final volume of 250 mL) adjusting the pH to 5.60 with 0.5 M NaOH. The stock solution of the enzyme was made by mixing 12.5 mg of the 0.25 U/mL acid phosphatase powder in 2.0 ml acetate buffer. For use, 100 μL of the stock solution was diluted with 1.9 mL acetate buffer. For the substrate solution 0.170 g of p-NPP were dissolved in 2.5 mL distilled water.

2.2.1. Test procedure

The compounds solutions were prepared by diluting the stock solutions prepared in DMSO with acetate buffer. A volume of 0.50 mL of complex solution was mixed with 0.10 mL of the enzyme solution and 1.00 mL of buffer. The mixture was kept at 25 °C for 20 min (incubation time). After starting the reaction by adding 0.10 mL of the substrate solution, the tube was kept at 25 °C for 20 min. The reaction was stopped with the addition of 0.50 mL of a 0.5 M sodium hydroxide solution. The final concentration of DMSO resulted in 1.14%. The enzymatic activity was finally calculated by measuring the absorbance of 4-nitrophenolate at 405 nm against a blank prepared without the enzyme. Three independent replicates of each point were measured. The 100% of the enzyme activity is assigned to a basal measurement containing all the reaction media including the same volume of DMSO in all the experiments. It is worthy to mention that the presence of 1.14% DMSO did not affect the enzyme activity.

2.3. Anti-thyroid activity

Iodine was obtained from Merck. It was bisublimed and was kept in dark in a desiccator containing P_2O_5. Solvents used were bidistilled water and ethanol. Solutions of iodine and methimazole, o-phenanthroline, Cu–Met and Cu–Met–phen were prepared just before the beginning of experimentation. Stock solutions: iodine was always dissolved in ethanol while the compounds were dissolved in water. Iodine concentration was kept constant (4×10^{-4} M), though the concentration of the compounds was varied between 7×10^{-4} and 0.5×10^{-4} M. The reaction was carried out directly in the spectrophotometric cell by mixing 1.5 mL of each of compound (donor) and iodine (acceptor). Spectra were recorded immediately on double beam UV–visible spectrophotometer. The temperature of the solutions was kept at $25^0 \pm 1$ °C during the measurements. Three independent replicates of each solution were measured. Formation constant (K_c) and the molar extinction coefficient were determined using Lang's method [22]. This method has been used to determine the formation constants of 1:1 stoichiometric complexes at the wavelength under analysis using Eq. (1):

$$[A_0][D_0]/d_c = ([A_0] + [D_0] - d_c/\varepsilon_c)/\varepsilon_c + 1/K_c\varepsilon_c \quad (1)$$

in which, d_c is the absorbance, ε_c is the molar extinction coefficient and K_c is the formation constant of the complex. The Parameters were adjusted with a program designed by our research group.

Eq. (1) can be re-written in the form:

$$Y = (1/\varepsilon_c)X + 1/K_c\varepsilon_c \quad (2)$$

where $Y = [A_0][D_0]/d_c$ and $X = [A_0] + [D_0] - d_c/\varepsilon_c$.

From Eq. (2) a straight line with slope $1/\varepsilon_c$ and Y-intercept $1/(K_c\varepsilon_c)$ was obtained. Iteration and linear regression method were used to solve this equation.

2.4. Antifungal activity and post-antifungal effect (PAFE)

Antifungal activity was evaluated by the determination of the lowest concentration of the antimicrobial agent that inhibits the growth of fungus (minimum inhibitory concentration (MIC)) on three strains: *Candida parapsilosis* ATCC 22019, *Candida tropicalis* and *Candida albicans* of clinical isolates. The procedure was

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