Journal of Inorganic Biochemistry 187 (2018) 62–72

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Complexes in aqueous cobalt(II)–2-picolinehydroxamic acid system: Formation equilibria, DNA-binding ability, antimicrobial and cytotoxic properties

Magdalena Woźniczka[a,*], Mirosława Świątek[a], Marek Pająk[a], Joanna Gądek-Sobczyńska[a], Magdalena Chmiela[b], Weronika Gonciarz[b], Paweł Lisiecki[c], Beata Pasternak[d], Aleksander Kufelnicki[a]

[a] Department of Physical and Biocoordination Chemistry, Faculty of Pharmacy, Medical University of Lodz, Muszyńskiego 1, 90-151 Lodz, Poland
[b] Department of Immunology and Infectious Biology, Institute of Microbiology, Biotechnology and Immunology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland
[c] Department of Pharmaceutical Microbiology and Microbiological Diagnostics, Medical University of Lodz, Pomorska 137, 90-235 Lodz, Poland
[d] Department of Organic Chemistry, Faculty of Chemistry, University of Lodz, Tamka 12, 91-403 Lodz, Poland

ARTICLE INFO

Keywords:
Cobalt(II) complexes
2-Picolinehydroxamic acid
pH potentiometry
UV-Vis spectroscopy
DNA interaction
Antimicrobial activity

ABSTRACT

The coordination properties of 2-picolinehydroxamic acid towards cobalt(II) in aqueous solution were determined by a pH-metric method and confirmed by spectroscopic (UV–Vis and ESI-MS) studies. The results show the formation of mononuclear complexes, as well as of metallacrowns (MC). All methods indicate a high tendency of 2-picolinehydroxamic acid to form cobalt(II) metallacrown 12-MC-4. ESI-MS additionally confirms 15-MC-5 and 18-MC-6, stabilized by a sodium ion and methanol. The complexes observed in the speciation model at a pH about 7.2 were studied for their DNA-binding ability. The decrease of absorbance in the range of *ca* 310–400 nm indicates effective binding to calf thymus DNA by 2-picolinehydroxamic acid complexes, *via* intercalative mode. The antimicrobial properties of 2-picolinehydroxamic acid, cobalt(II) ions and of the complexes formed in the Co(II) – ligand system were determined against Gram-positive bacteria (*Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Helicobacter pylori*) and fungal strains (*Candida*, *Aspergillus niger*). The results indicate that the complexes demonstrate greater antibacterial and antifungal activity for most strains than the ligand. Both the complexes and the ligand induce a slight decrease in the metabolic activity of cells, while the complexes do not damage the cell nuclei. The 2-picolinehydroxamic acid complexes activate the human monocytic cells, suggesting they have immunomodulating properties, which are particularly important in combating infections caused by strains resistant to other drugs.

1. Introduction

Hydroxamic acids are known as reagents for many metal ions in coordination and supramolecular chemistry. They can affect biological activity as growth factors, antibiotics and specific metalloenzyme inhibitors [1]. The inhibition effect is related to chelation of the catalytic metal center by hydroxamate groups [2, 3]. Thanks to their therapeutic potential, they are known as inhibitors of DNA biosynthesis and siderophores used in the chelation therapy of iron and aluminium overload diseases [4–6]. In addition, hydroxamic acids show antihypertensive, anticancer, antimalarial and antifungal properties [7].

Hydroxamic acids are often used as bridging ligands in the formation of metallacrowns with a repetitive $-[M-N-O]_n-$ unit in the cyclic system, which affects their stable structures in various solvents and in the gas phase [8–10]. The metallacrowns are characterized by the presence of an oxygen-rich core cavity encapsulating cations or anions [11]. These compounds are interesting because of their potentially unique properties as luminescent materials and selective cation or anion recognition agents [12]. In the case of copper metallacrowns with hydroxamic acid derivatives, an ability to bind anions and encapsulate metal ions important in magnetic resonance imaging (MRI) or radiotherapy is observed [13]. In turn, the manganese and nickel

* Corresponding author.
E-mail address: magdalena.wozniczka@umed.lodz.pl (M. Woźniczka).

https://doi.org/10.1016/j.jinorgbio.2018.07.010
Received 5 April 2018; Received in revised form 20 June 2018; Accepted 19 July 2018
Available online 24 July 2018

metallacrowns, containing salicylhydroxamic ligands, exhibit antibacterial activity [9, 14, 15].

In recent years, there has been considerable interest in the interactions of transition metal complexes with DNA and their potential biological and medical applications, particularly in chemotherapy. A huge number of studies focused on the binding mode of metal complexes to deoxyribonucleic acid, mainly by intercalation with π-π stacking and the electrostatic interactions occurring between the positive charges on the intercalator and the phosphates of DNA outside the helix [16–19]. Another possibility of the electrostatic binding exists between the electron-rich groups of the ligand and the base pairs of DNA [18]. As a result of these bonds, the DNA double helix is stabilized, lengthened, stiffened, unwound and subjected to cleavage. These important changes in DNA can form the basis of antibacterial and anticancer activity. Hydroxamic acid complexes are able to promote such interactions [15]. They contain the smallest C═ONHOH units that can bind to the DNA helix, allowing them to be used in drug design [4].

Complexes with several *d*-block transition metals such as Cu(II), Zn (II), Ni(II) and Mn(II) have a well-studied effect on DNA [8, 20, 21]. However, there are also reports regarding the DNA-binding properties of cobalt(II) complexes [22]. Cobalt(II) is a biologically important metal, its complexes are known for their biochemical roles. Apart from vitamin B_{12}, cobalt(II) is included also in enzymes: prolidase, glucose isomerase, lysine-2,3-aminomutase, methylmalonyl-CoA carboxytransferase, bromoperoxidase and methionine aminopeptidase [23, 24].

The present study examines the complexation equilibria for the cobalt(II) – 2-picolinehydroxamic acid (PicHA, Fig. 1) system in aqueous solution. It is known that 2-picolinehydroxamic acid, an α-aminohydroxamic acid representative, is able to bridge metal ions and create various polynuclear species, which makes it one of the most used hydroxamic acids in metallacrowns synthesis [25, 26]. The presence of such a wide range of coordination modes prompted this study, especially since the complexes of this ligand with Cu(II), Ni(II) and Zn(II) have previously also been characterized in aqueous solutions [11].

The present study also examines the biological properties of 2-picolinehydroxamic acid and its cobalt(II) complexes in aqueous solutions, of which little is known. The DNA-binding abilities of the 2-picolinehydroxamic acid complexes were studied by measuring their effect on the UV–Vis spectra of the substance. A strongly flattened ligand conformation is preferred for DNA binding, as is the presence of a pyridine ring, which enables π-π stacking interactions in crystal structures [18, 27].

The increasing antibiotic resistance of bacterial pathogens to commonly-used antibiotics has encouraged an intensive search for new antibacterial preparations with therapeutic abilities [28–30]. Chemically synthesized compounds containing metal ions have been found to demonstrate antimicrobial and antifungal potential [31, 32].

The purpose of the present study was to evaluate the antimicrobial properties of ionic forms of PicHA, as well as complexes present in the aqueous Co(II) – PicHA system, towards several reference bacterial and fungal pathogens that cause serious health complications in humans. In addition, the cytotoxicity of the formulations was determined using the MTT reduction assay and DAPI staining of standard L929 mouse fibroblasts. As non-specific immune cells also play a role in infection control, the study also examines whether the formulations used are able to stimulate the human monocytic cells as an indirect form of antimicrobial activity.

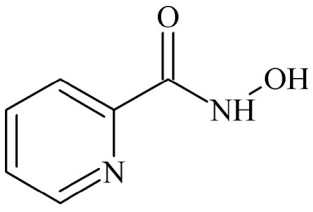

Fig. 1. Structure of the ligand 2-picolinehydroxamic acid (PicHA, LH).

2. Experimental

2.1. Materials

2-Picolinehydroxamic acid (PicHA) was synthesized by a team at the Department of Chemistry, National Taras Shevchenko University of Kiev [33]. Cobalt(II) nitrate hexahydrate and cobalt(II) perchlorate hexahydrate from Sigma-Aldrich were used as standard solutions; these were titrated with disodium salt of EDTA in the presence of murexide. The carbonate-free 0.1 M and 1.0 M NaOH solutions, methanol and water (HPLC-grade) were purchased from J.T. Baker. The solutions of the mineral acids (HNO_3 and $HClO_4$ from Sigma-Aldrich) were standardized alkalimetrically and determined by the Gran method [34]. The following standard solutions were used to adjust the ionic medium without further purification: potassium nitrate(V) (J.T. Baker) and sodium perchlorate monohydrate (Sigma-Aldrich). DNA sodium salt from calf thymus and Tris-HCl were obtained from Sigma-Aldrich. Sodium chloride (Chempur) and argon of high purity (Linde) were used.

The following materials were used in the biological studies: Mueller-Hinton broth (bioMeuriex), horse blood lysate (Biomed), *β*-NAD, RPMI-1640, amphotericin B, gentamicin, amoxicillin, penicillin, streptomycin, trypsin, MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)], 4′,6-diamidino-2-phenylindole (DAPI), *E. coli* serotype O55:B5 lipopolysaccharide (LPS) (all from Sigma-Aldrich), Brain Heart Infusion Agar (Becton Dickinson), fetal bovine serum (FBS) (Cytogen), Quanti-Blue reagent, normocin and zeocin (all from Invitrogen).

2.2. Potentiometric measurements

Equilibrium studies were carried out at a constant temperature of 25.0 ± 0.1 °C using a Titrando 905 automatic titrator system (Metrohm) with a 800 Dosino unit and a combined Metrohm LL Biotrode filled with 3 M KCl electrolyte of the reference electrode. All the experiments were performed in aqueous solutions as described previously [35, 36] at ionic strength $I = 0.1$ M (KNO_3). The protonation constants were determined for different concentrations of the ligand (2×10^{-3}–10^{-2} M) in the pH range 1.6–11.0. Titrations in the presence of Co(II) were carried out at ligand-to-metal molar ratios 1.5:1, 2:1, 3:1, 4:1 (at constant concentration of PicHA 10^{-2} M) in the pH range 1.8–11.0. Purified argon was continuously passed over the solution surface in the titration vessel to ensure the absence of oxygen and carbon dioxide. The hydrolysis constants of cobalt(II) were determined under the same conditions.

Overall concentration formation constants were calculated by the Hyperquad 2008 fitting procedure according to the formula: $\beta_{mlh} = [M_mL_lH_h] / [M]^m[L]^l[H]^h$, using the nonlinear least-squares method [37]. The distribution curves of the complexation species were calculated as a function of pH using the HySS 2009 [38].

2.3. Electrospray-ionization mass spectrometry (ESI-MS) measurements

The ESI-MS spectra were recorded using a Varian 500-MS LC hexapole ion-trap mass spectrometer (Palo Alto, CA, USA). The experiments were performed for PicHA and Co(II) – PicHA system in 50/50% (v/v) methanol/water mixture without the addition of a background electrolyte. The ligand concentration was 10^{-2} M in all the samples. For Co(II) – ligand system, the molar ligand-metal ratios were equal to 2:1 and 3:1. The samples were acidified (HNO_3) or alkalized (NaOH) to various pH values in order to allow for different types of complexes (based on potentiometric species distribution graphs). All samples were introduced into the ESI-MS source by continuous infusion using an instrument syringe pump at a rate of 10 μL min^{-1}. The ESI-