Journal of Inorganic Biochemistry 121 (2013) 53–65

Contents lists available at SciVerse ScienceDirect

Journal of Inorganic Biochemistry

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

First- and second-generation quinolone antibacterial drugs interacting with zinc(II): Structure and biological perspectives

Alketa Tarushi [a], Kostas Lafazanis [b], Jakob Kljun [c], Iztok Turel [c], Anastasia A. Pantazaki [b], George Psomas [a], Dimitris P. Kessissoglou [a,*]

[a] Laboratory of Inorganic Chemistry, Faculty of Chemistry, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece
[b] Laboratory of Biochemistry, Faculty of Chemistry, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece
[c] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Askerceva 5, 1000 Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 8 October 2012
Received in revised form 15 December 2012
Accepted 15 December 2012
Available online 23 December 2012

Keywords:
Zinc complexes
Quinolones
Interaction with DNA
Competitive studies with ethidium bromide
Interaction with serum albumins
Antimicrobial activity

ABSTRACT

Interaction of equimolar quantities of $ZnCl_2$ with the quinolone antibacterial drugs flumequine (Hflmq), oxolinic acid (Hoxo) or enrofloxacin (Herx) and the N,N′-donor heterocyclic ligands 1,10-phenanthroline (phen) or 2,2′-bipyridine (bipy) results in the formation of 1:1 drug to metal complexes with the general formula [Zn(quinolone)(N,N′-donor)Cl], while excess of the quinolone leads to 1:2 metal to drug [Zn(quinolone)$_2$(N,N′-donor)] complexes. In all complexes, the deprotonated bidentate quinolonato ligands are coordinated to zinc ion through the pyridone oxygen and a carboxylato oxygen. The crystal structures of [Zn(oxo)(phen)Cl], [Zn(flmq)(phen)Cl] and [Zn(flmq)$_2$(phen)] have been determined by X-ray crystallography. All complexes exhibit good binding propensity to human or bovine serum albumin protein showing relatively high binding constant values. Interaction of the complexes with calf-thymus (CT) DNA, studied by UV spectroscopy, has shown that they bind to CT DNA, while [Zn(flmq)(phen)Cl] and [Zn(flmq)$_2$(phen)] complexes exhibit the highest binding constants to CT DNA. Competitive study with ethidium bromide (EB) has shown that all complexes can displace the DNA-bound EB indicating that they bind to DNA in strong competition with EB. Intercalative binding mode is proposed for the interaction of the complexes with CT DNA and has also been verified by DNA solution viscosity measurements. DNA electrophoretic mobility experiments suggest that all complexes bind to linearized pDNA and supercoiled pDNA by intercalative manner resulting in catenanes formation as well as in double-stranded cleavage reflecting (or ending) in the formation of linear DNA. The complexes exhibit significant antimicrobial activity tested on five different microorganisms.

1. Introduction

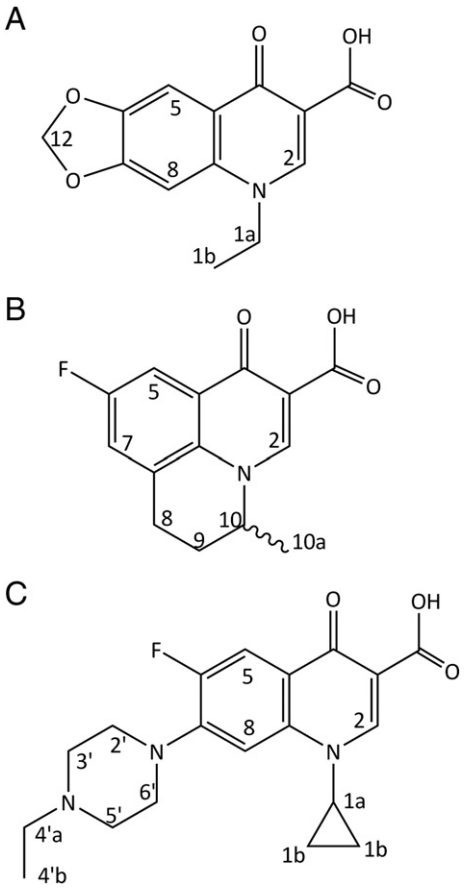
Quinolones (quinolonecarboxylic acids or 4-quinolones), a group of synthetic antibacterial agents containing a 4-oxo-1,4-dihydroquinoline skeleton, are commonly used in the treatment of many infections [1,2]. Their main targets are gyrases (type II topoisomerases) and topoisomerase IV, enzymes that participate in the DNA replication [3]. Interaction of quinolones with DNA [4], antibacterial activity tests on diverse microorganisms, cytotoxicity [5] and potential antitumor activity [6] are among the biological properties thoroughly studied. In many cases, the metal complexes of drugs are more active than their parent compounds [7,8]. From this point of view, diverse metal complexes with quinolones have been synthesized in an attempt to investigate the physicochemical properties and to evaluate their biological properties (antibacterial activity [9,10], interaction with DNA [11–17] and potential antitumor activity [15–17]) in comparison to free quinolones [1].

Oxolinic acid (Hoxo, (Scheme 1(A)) and flumequine, (Hflmq (Scheme 1(B)), first-generation quinolone antimicrobial drugs [1], are in use for the treatment of urinary tract infections and in veterinary medicine for the treatment of animal diseases caused by a wide-range of Gram-negative bacteria, respectively [18]. Although the pharmaceutical role of oxolinic acid and flumequine, are known for more than thirty years [19–21], only a few of their complexes with Cu(II), Zn(II) and Ni(II) metal ions have been structurally characterized, all reported earlier by our group [22–26]. Enrofloxacin (Herx, Scheme 1(C)) is a typical second-generation quinolone antimicrobial drug and presents a broad spectrum of activity against a wide range of Gram-negative and Gram-positive bacteria [1,18]. Enrofloxacin is the first fluoroquinolone developed for veterinary application and is usually used in the treatment of some urinary tract, respiratory tract and skin infectious diseases in pets and livestock [27]. Fe(III), Ni(II), Zn(II) and Cu(II) enrofloxacin complexes, all but one have been recently reported by our labs [28–33], while dinuclear Cd(II), Pb(II), La(III) and Sm(II) complexes have also been reported [34,35].

Besides the significant biological role of zinc [36,37], zinc compounds have been extensively used in children suffering from deadly

* Corresponding author. Tel.: +30 2310997723; fax: +30 2310997738.
E-mail address: kessisog@chem.auth.gr (D.P. Kessissoglou).

0162-0134/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jinorgbio.2012.12.009



Scheme 1. The quinolone ligands (A) oxolinic acid (=Hoxo), (B) flumequine (=Hflmq) and (C) enrofloxacin (=Herx) and H atoms labeling.

diarrhea in the form of "Baby Zinc" resulted in significant reduction of child mortality in countries of Asia and Africa [38]. Zinc complexes with drugs has been tested for the treatment of Alzheimer disease [39], while antibacterial [40], anticonvulsant [41], antidiabetic [42], antiinflammatory [43,44], antimicrobial [45] and antiproliferative-antitumor [44,45] activity has been shown for structurally characterized compounds.

Taking into consideration the biological role and the activity of zinc complexes, the significance of quinolones in medicine and the fact that metal complexes with drugs may usually exhibit more pronounced biological properties in comparison to the free drugs, we have already initiated studies on the interaction of zinc(II) with quinolone antimicrobial drugs, oxolinic acid, flumequine and enrofloxacin in the presence of the N,N′-donor heterocyclic ligands 2,2′-bipyridine (bipy) and 1,10-phenanthroline (phen) in an attempt to investigate the biological properties of the resultant complexes [23,29,30] and possible synergetic effects. In this context, we report the synthesis, characterization and structure of the mononuclear complexes [Zn(oxo)(phen)Cl]·MeOH **(1**·MeOH), [Zn(flmq)(phen)Cl]·0.5MeOH, **(2**·0.5MeOH), [Zn(erx)(phen)Cl] **(3)**, [Zn(erx)(bipy)Cl] **(4)**, [Zn(flmq)$_2$(phen)]·MeOH, (**5**·MeOH) and [Zn(oxo)$_2$(bipy)] (**6**). The crystal structures of [Zn(oxo)(phen)Cl]·MeOH, [Zn(flmq)(phen)Cl]·0.5MeOH and [Zn(flmq)$_2$(phen)]·MeOH have been determined by X-ray crystallography. Furthermore, our studies have been focused on (i) the affinity to bovine (BSA) and human serum albumin (HSA) – proteins involved in the transport of metal ions and metal–drug complexes through the blood stream – investigated by fluorescence spectroscopy, (ii) the binding strength of the complexes with calf-thymus (CT) DNA investigated by UV spectroscopy and viscosity measurements, (iii) the competitive binding studies with ethidium bromide (EB) performed by fluorescence spectroscopy in order to investigate the existence of a potential intercalation of the complexes to CT DNA, (iv) the binding and the nuclease-like activity of the complexes on linear and pDNA studied by agarose gel electrophoresis and (v) the antimicrobial activity of the complexes by determining the minimum inhibitory concentration (MIC) against five Gram-positive or Gram-negative microorganisms. Additionally, the affinity of the previously reported Zn(II) oxolinato complexes [Zn(oxo)$_2$(H$_2$O)$_2$] (**7**), [Zn(oxo)(bipy)Cl] (**8**) and [Zn(oxo)$_2$(phen)] (**9**) to BSA and HSA has been evaluated.

2. Experimental

2.1. Materials — instrumentation — physical measurements

All chemicals (oxolinic acid, flumequine, enrofloxacin, $ZnCl_2$, 1,10-phenanthroline, 2,2′-bipyridine, KOH, NaCl, trisodium citrate, CT DNA, BSA, HSA and EB) were purchased from Sigma-Aldrich Co and all solvents from Merck. All chemicals and solvents were reagent grade and were used as purchased.

Agarose for electrophoresis was purchased from BRL. Tryptone and yeast extract for antimicrobial activity were purchased from Oxoid (Unipath Ltd., Hampshire, UK). The intercalative dye EB was purchased from Sigma. Plasmid DNA, pDNA (pET29c) was isolated from *Escherichia coli* (Top 10) using the GenEluteTM HP endotoxin-free plasmid maxiprep preparation (Sigma–Aldrich), according to the manufacturer's specifications. For creation of linear DNA, pDNA was digested using Hind III restriction endonuclease (Amersham) according to the manufacturer's instructions.

DNA stock solution was prepared by dilution of CT DNA to buffer (containing 150 mM NaCl and 15 mM trisodium citrate at pH 7.0) followed by exhaustive stirring at 4 °C for three days, and kept at 4 °C for no longer than a week. The stock solution of CT DNA gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of ~1.9, indicating that the DNA was sufficiently free of protein contamination. The DNA concentration was determined by the UV absorbance at 260 nm after 1:20 dilution using $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [8].

Infrared (IR) spectra (400–4000 cm^{-1}) were recorded on a Nicolet FT-IR 6700 spectrometer with samples prepared as KBr disk. UV-visible (UV-vis) spectra were recorded as nujol mulls and in solution at concentrations in the range 10^{-5}–10^{-3} M on a Hitachi U-2001 dual beam spectrophotometer. ^{1}H-NMR spectra were measured at room temperature on a Bruker AM300 NMR spectrometer using d_6-DMSO or d_6-DMSO/$CDCl_3$ (ratio 5:1) as solvent. C, H and N elemental analysis were performed on a Perkin-Elmer 240B elemental analyzer. Molar conductivity measurements of 1 mM dimethylsulfoxide (DMSO) solutions of the complexes were carried out with a Crison Basic 30 conductometer. Fluorescence spectra were recorded in solution on a Hitachi F-7000 fluorescence spectrophotometer. Viscosity experiments were carried out using an ALPHA L Fungilab rotational viscometer equipped with an 18 mL LCP spindle.

2.2. Synthesis of the complexes

2.2.1. [Zn(oxo)(phen)Cl]·MeOH, 1·MeOH

Oxolinic acid (0.4 mmol, 105 mg) dissolved in CH_3OH (10 mL) was deprotonated by KOH (0.4 mmol, 22 mg). The resultant solution was stirred for about 20 min at ambient temperature. The solution of deprotonated oxolinic acid and a methanolic solution (5 mL) of phen (0.4 mmol, 72 mg) were added simultaneously dropwise to a methanolic solution (5 mL) of $ZnCl_2$ (0.4 mmol, 56 mg). The reaction mixture was stirred for about 1 h and left for slow evaporation. Colorless crystals of [Zn(oxo)(phen)Cl]·MeOH, **1**·MeOH, suitable for X-ray structure determination were collected after a few days. Yield: 170 mg, 75%. *Anal.* Calcd. for [Zn(oxo)(phen)Cl]·MeOH ($C_{26}H_{22}N_3O_6ClZn$) (MW = 573.31): C 54.47, H 3.87, N 7.33; found C 54.31, H 4.05, N 6.95. IR (KBr pellet): ν_{max}/cm^{-1} $\nu(C=O)_{pyridone}$ 1641(very strong (vs)); $\nu_{asym}(CO_2)$: 1597(vs); $\nu_{sym}(CO_2)$: 1392(vs);