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Ni(II) complexes with non-steroidal anti-inflammatory drug diclofenac: Structure and interaction with DNA and albumins

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ABSTRACT

The interaction of nickel(II) with the non-steroidal anti-inflammatory drug sodium diclofenac (Nadicl) in the presence of the N,N′-donor heterocyclic ligands 2,2′-dipyridylketone oxime (Hpko), 2,2′-bipyridine (bipy) or 1,10-phenanthroline (phen) leads to the formation of mononuclear Ni(II) complexes. The crystal structure of $[Ni(dicl)(Hdicl)(Hpko)_2](dicl)\cdot CH_3OH\cdot 0.6H_2O$ (**1**·$CH_3OH\cdot 0.6H_2O$) has been determined by X-ray crystallography. The interaction of the complexes with human or bovine serum albumins has been studied by fluorescence spectroscopy revealing their good binding affinity to the albumins with high binding constant values. UV study of the interaction of the complexes with calf-thymus DNA (CT DNA) has shown that the complexes can bind to CT DNA with $[Ni(dicl)(Hdicl)(Hpko)_2](dicl)$ exhibiting the highest binding constant to CT DNA. Complex **1** can bind to CT DNA via intercalation as concluded by studying its cyclic voltammograms in the presence of CT DNA solution and by DNA solution viscosity measurements, while for complexes $[Ni(dicl)_2(bipy)]$ (**2**) and $[Ni(dicl)_2(phen)]$ (**3**) a non-classic intercalative mode has been concluded. Competitive studies of the complexes with ethidium bromide (EB) have shown their moderate to significant ability to displace the DNA-bound EB suggesting a competition with EB.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently used medical drugs as analgesics, anti-inflammatories and antipyretics [1]. NSAIDs act by inhibiting the production of prostaglandins which is mediated by cyclo-oxygenase (COX) [2]. NSAIDs present antitumorigenic properties attributed to COX-independent mechanisms [3,4], to apoptosis via an activation of caspases [5] or via an unknown molecular mechanism where free radical may be involved [6]. Thus, the interaction of NSAIDs and their complexes with DNA should be considered of great importance and further evaluated as a means to the potential anticancer activity since few relevant reports on the interaction of NSAIDs and their complexes with DNA have been published so far [7,8]. The chemical classes of NSAIDs comprise salicylate derivatives, phenylalkanoic acids, oxicams, anthranilic acids, sulfonamides and furanones [9]. Sodium diclofenac (=Nadicl, Scheme 1) is a potent NSAID of the phenylalkanoic acids' group exhibiting favorable anti-inflammatory, analgesic and antipyretic properties [10]. It is mainly used in painful and inflammation conditions like rheumatoid arthritis, spondilytis, and osteoarthritis. This drug inhibits COX activity *in vitro* with no significant effect on phospholipase A_2 or on lipoxygenase enzymes [11]. In the literature, the crystal structures of three copper(II) [12–14], a cadmium(II) [15] and a tin(IV) [16] complexes with diclofenac ligands have been found.

The discovery in 1975 that urease is a nickel enzyme [17] was the first evidence of the important role of nickel in biological systems. Since then, the role of Ni has been largely expanded, not only due to the determination and the significant increase of the number of nickel-dependent or nickel-containing enzymes [18,19], but also because of the plethora of reported nickel complexes showing biological activity. Nickel complexes have been reported to act as anticonvulsant [20], antiepileptic [21] agents or vitamins [22]; they have also presented antibacterial [23,24], antifungal [24,25], antimicrobial [26,27], antioxidant [28] and antiproliferative/anticancer [29,30] activities. Furthermore, the interaction of Ni(II) complexes with biomolecules such as serum albumins [27,31–33] or DNA [31–36] has been recently studied revealing that the binding to these molecules and its mode is mainly dependent on

Abbreviations: Bipy, 2,2′-bipyridine; BSA, bovine serum albumin; COX, cyclo-oxygenase; CT, calf-thymus; DMF, N,N-dimethylformamide; EB, ethidium bromide, 3,8-diamino-5-ethyl-6-phenyl-phenanthridinium bromide; HSA, human serum albumin; Nadicl, sodium diclofenac, sodium 2-(2,6-dichlorophenylamino)phenyl-acetate); NSAID, non-steroidal anti-inflammatory drug; phen, 1,10-phenathroline; s, strong; SA, serum albumin; sh, shoulder; TEAP, tetraethylammonium perchlorate; vs, very strong; Δ, $\nu_{asym}(CO_2) - \nu_{sym}(CO_2)$.

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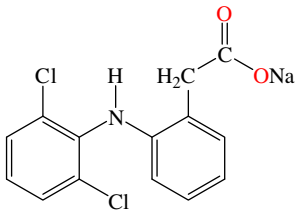

Scheme 1. Sodium diclofenac (=Nadicl).

the structure of the ligand [37]. A thorough survey of the literature concerning Ni(II) complexes with NSAIDs has revealed few reports [38–41] describing the isolation of such complexes without the determination of any X-ray crystal structure.

Given the increasing biological interest in metal complexes and especially nickel compounds as well the significance of NSAIDs in medicine, we report the synthesis, the structural and spectroscopic characterization and the biological properties of nickel(II) complexes with the NSAID sodium diclofenac in the presence of a N,N′-donor heterocyclic ligand such as 2,2′-dipyridylketone oxime (=Hpko), 2,2′-bipyridine (=bipy) or 1,10-phenanthroline (=phen). The interaction of Ni(II) with Nadicl in the presence of Hpko leads to the formation of complex $[Ni(dicl)(Hdicl)(Hpko)_2](dicl)\cdot CH_3OH\cdot 0.6H_2O$ (=**1**$\cdot CH_3OH\cdot 0.6H_2O$) that has been structurally characterized by X-ray crystallography, while in the presence of bipy or phen $[Ni(dicl)_2(bipy)]$ (=**2**) or $[Ni(dicl)_2(phen)]$ (=**3**) complexes have been isolated, respectively. The complexes have been characterized with physicochemical and spectroscopic techniques and their electrochemical behavior has been also investigated. In order to investigate the possibility of existence of any potential biological activity of complexes **1**–**3**, the study of the biological properties of the complexes has been focused on (i) their binding properties with CT DNA investigated by UV spectroscopy, cyclic voltammetry and viscosity measurements, (ii) the ability to displace ethidium bromide (=EB) from the EB–DNA complex, as a means to clarify the existence of a potential intercalation of the complexes to CT DNA in competition to the classical DNA-intercalator EB, performed by fluorescence spectroscopy and (iii) the affinity of the complexes to bovine (=BSA) and human serum albumin (=HSA) – binding to these proteins involved in the transport of metal ions and metal-drug complexes through the blood stream may reveal lower or enhanced biological properties of the original drug, or new paths for drug transportation [42] – investigated by fluorescence spectroscopy.

2. Experimental

2.1. Materials – instrumentation – physical measurements

The chemical reagents $NiCl_2\cdot 6H_2O$, sodium diclofenac, phen, bipy, Hpko, KOH, CT DNA, BSA, HSA, EB, tetraethylammonium perchlorate (TEAP), NaCl and trisodium citrate were purchased from Sigma–Aldrich Co. and all solvents were purchased from Merck. All chemicals and solvents were reagent grade and were used as purchased without any further purification. TEAP was recrystallized twice from ethanol, prior to its use, and dried under vacuum.

DNA stock solution was prepared by dilution of CT DNA to buffer (containing 15 mM trisodium citrate and 150 mM NaCl at pH 7.0) followed by exhaustive stirring 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 1.87, 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 $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [12].

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. Room temperature magnetic measurements were carried out by the Faraday method. C, H and N elemental analysis were performed on a Perkin–Elmer 240B elemental analyzer. Molar conductivity measurements were carried out with a Crison Basic 30 conductometer. Fluorescence spectra were recorded in solution on a Hitachi F-7000 fluorescence spectrophotometer.

Cyclic voltammetry studies were performed on an Eco chemie Autolab Electrochemical analyzer. Cyclic voltammetry experiments were carried out in a 30 mL three-electrode electrolytic cell. The working electrode was platinum disk, a separate Pt single-sheet electrode was used as the counter electrode and a Ag/AgCl electrode saturated with KCl was used as the reference electrode. The cyclic voltammograms of the complexes were recorded in 0.4 mM DMSO solutions and in 0.4 mM 1/2 DMSO/buffer solutions at $v = 100\ mV\ s^{-1}$ where TEAP and the buffer solution were the supporting electrolytes, respectively. Oxygen was removed by purging the solutions with pure nitrogen which had been previously saturated with solvent vapors. All electrochemical measurements were performed at 25.0 ± 0.2 °C.

2.2. Synthesis of the complexes

2.2.1. *[Ni(dicl)(Hdicl)(Hpko)$_2$](dicl)·CH$_3$OH·0.6H$_2$O,* **1**·*CH$_3$OH·0.6H$_2$O*

A methanolic solution (10 mL) of Nadicl (126 mg, 0.4 mmol) and a methanolic solution (5 mL) of Hpko (79 mg, 0.4 mmol) were added simultaneously and dropwise in a methanolic solution (10 mL) of $NiCl_2\cdot 6H_2O$ (47 mg, 0.2 mmol). The resultant solution was stirred for 5 min and left for slow evaporation. Light-green crystals of $[Ni(dicl)(Hdicl)(Hpko)_2](dicl)\cdot CH_3OH\cdot 0.6H_2O$, **1**$\cdot CH_3OH\cdot 0.6H_2O$, suitable for X-ray structure determination were collected after a couple of days. Yield: 180 mg, 65%. *Anal.* Calc. for $[Ni(dicl)(Hdicl)(Hpko)_2](dicl)\cdot CH_3OH\cdot 0.6H_2O$ ($C_{65}H_{54.2}Cl_6N_9NiO_{9.6}$) (MW = 1386.38): C, 56.31; H, 3.94; N, 9.09. Found: C, 56.25; H, 3.79; N, 8.85%. IR (KBr disk): ν_{max}, cm^{-1}; $\nu(C{=}O)_{carboxylic}$: 1708 (very strong (vs)); $\nu_{asym}(CO_2)$: 1591 (vs); $\nu_{sym}(CO_2)$: 1387 (vs); $\nu(C{-}O)_{carboxylic}$: 1281 (s); $\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2) = 204\ cm^{-1}$; UV–Vis: λ, nm (ε, $M^{-1}\ cm^{-1}$) as Nujol mull: 975, 575, 398(sh), 350, 304(sh); in DMSO: 960 (13), 590 (160), 405(sh) (375), 346 (1500), 307 (1900); 10Dq = 10415 cm^{-1}, $B = 693\ cm^{-1}$. $\mu_{eff} = 2.87$ BM. The complex is soluble in DMSO ($\Lambda_M = 20$ mho $cm^2\ mol^{-1}$, in 1 mM DMSO) and partially soluble in DMF, ethanol and chloroform.

2.2.2. *[Ni(dicl)$_2$(B)] (B = bipy for* **2***, phen for* **3***)*

Complexes **2** and **3** have been prepared in a similar way to complex **1** with the use of the corresponding N,N′-donor ligand (B), bipy (0.2 mmol, 31 mg) for **2** and phen (0.2 mmol, 36 mg) for **3**. Pale green microcrystalline product of $[Ni(dicl)_2(bipy)]$ and $[Ni(dicl)_2(phen)]$, respectively, was collected after a few days.

Data for **2**: Yield: 120 mg, 75%. *Anal.* Calc. for $[Ni(dicl)_2(bipy)]$ ($C_{40}H_{28}Cl_4N_4NiO_4$) (MW = 805.19): C, 56.69; H, 3.51; N, 6.96. Found: C, 56.51; H, 3.38; N, 6.77%. IR (KBr disk): ν_{max}, cm^{-1}; $\nu_{asym}(CO_2)$: 1576 (vs); $\nu_{sym}(CO_2)$: 1423 (vs); $\Delta = 153\ cm^{-1}$; UV–Vis: λ, nm (ε, $M^{-1}\ cm^{-1}$) as Nujol mull: 1010, 605, 390(sh), 315(sh), 295; in DMSO: 995 (15), 609 (25), 395(sh) (160), 310(sh) (1800), 296 (6500); 10Dq = 10050 cm^{-1}, $B = 772\ cm^{-1}$. $\mu_{eff} = 3.01$ BM. The complex is soluble in ethanol and DMSO ($\Lambda_M = 8$ mho $cm^2\ mol^{-1}$, in 1 mM DMSO) and partially soluble in DMF.

Data for **3**: Yield: 115 mg, 70%. *Anal.* Calc. for $[Ni(dicl)_2(phen)]$ ($C_{40}H_{28}Cl_4N_4NiO_4$) (MW = 829.21): C, 57.94; H, 3.40; N, 6.76. Found: C, 58.25; H, 3.49; N, 6.95%. IR (KBr disk): ν_{max}, cm^{-1}; ν_{asym}