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Structure, DNA- and albumin-binding of the manganese(II) complex with the non-steroidal antiinflammatory drug niflumic acid

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ABSTRACT

The manganese(II) complex with the non-steroidal antiinflammatory drug niflumic acid has been synthesized and characterized. The crystal structure of the complex [Mn(O-niflumato)$_2$(methanol)$_4$] has been determined by X-ray crystallography, where a monodentate coordination of niflumato ligand was revealed. Niflumic acid and its Mn(II) complex exhibit good binding affinity to human or bovine serum albumin proteins with high binding constant values. UV study of the interaction of the compounds with calf-thymus DNA (CT DNA) has shown that the compounds can bind to CT DNA and [Mn(O-niflumato)$_2$(-methanol)$_4$] exhibits higher binding constant to CT DNA than free niflumic acid. The compounds can bind to CT DNA via intercalation as concluded by DNA solution viscosity measurements. Competitive studies with ethidium bromide (EB) have shown that the compounds can displace the DNA-bound EB suggesting strong competition with EB.

1. Introduction

Non-steroidal antiinflammatory drugs (NSAIDs), among the most used analgesic, antiinflammatory and antipyretic agents [1], act through inhibition of the cyclo-oxygenase (COX)-mediated production of prostaglandins [2], while they have also presented a synergistic role on the activity of certain antitumor drugs leading a series of cancer cell lines to cell death via apoptosis [3]. As a means to explain the tentative anticancer and antiinflammatory activity of the NSAIDs and their complexes, the interaction with DNA is considered of great importance and should be further evaluated, although few relevant reports on the interaction of NSAIDs and their complexes with DNA have been published so far [4,5].

The chemical classes of NSAIDs comprise phenylalkanoic acids, salicylate derivatives, anthranilic acids, oxicams, sulfonamides and furanones [6]. Niflumic acid (=Hnif, Scheme 1) is a NSAID of N-phenylanthranilic acid derivatives and resembles chemically to mefenamic, tolfenamic and flufenamic acid and other fenamates in clinical use [6]. Hnif is used to treat inflammatory rheumatoid diseases and relieve acute pain and it is effective against period pains, pain after surgery, and fever [7,8]. The crystal structures of two dinuclear [8,9] and two mononuclear copper(II) complexes [10,11] and a silver(I) [12] complex of niflumic acid have been reported in the literature.

Manganese, one of the most significant biometals, is found in the active center of many enzymes of diverse functionality [13,14]. It is known that, like many other metal ions, also hydrated manganese(II) ions interact with DNA [15]. The crystal structure of oligonucleotide in the presence of manganese(II) revealed among other the involvement of the metal in the formation of cross-links between neighbor duplexes [16]. The crystal structure of nucleosome core particle has shown that manganese(II) interacts with N7 atom of guanines and adenines [17]. Manganese-containing compounds SC-52608 and Teslascan are used in medicine as anticancer and MRI contrast agents, respectively [18], and an increasing number of manganese complexes exhibit biological interest showing antibacterial [19,20], anticancer [21–23] and antifungal [24] activity. Furthermore, a thorough search of the literature has not revealed any structurally characterized manganese complexes with NSAIDs.

The interaction of carboxylate-containing non-steroidal antiinflammatory drugs with Co^{2+}, Cu^{2+} and Zn^{2+} [25–32] has been the subject of our recent studies covering the characterization of the resultant complexes and the interaction of these metal complexes with biomolecules such as DNA and serum albumin proteins, in an attempt to examine their mode of binding and possible biological relevance. Having in mind the significance of NSAIDs in medicine, the activity of manganese complexes and potential synergetic

Abbreviations: bipy, 2,2′-bipyridine; CT, calf-thymus; bipyam, 2,2′-bipyridylamine; BSA, bovine serum albumin; COX, cyclo-oxygenase; DMF, N,N-dimethylformamide; DMSO, dimethylsulfoxide; EB, ethidium bromide, 3,8-diamino-5-ethyl-6-phenyl-phenanthridinium bromide; Hnif, niflumic acid, 2-[3-(trifluoromethyl)anilino]nicotinic acid; HSA, human serum albumin; NSAID, non-steroidal antiinflammatory drug; phen, 1,10-phenanthroline; py, pyridine; SA, serum albumin; sh, shoulder; vs, very strong; Δ, $\nu_{asym}(CO_2)-\nu_{sym}(CO_2)$.

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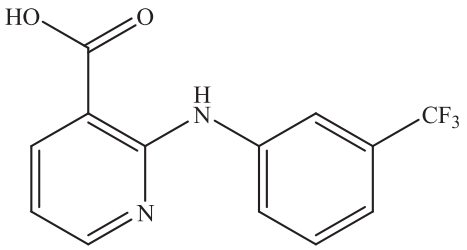

Scheme 1. Niflumic acid (=Hnif).

effects, we present the synthesis and the structural characterization of the mononuclear Mn(II) complex with the NSAID niflumic acid $[Mn(nif)_2(MeOH)_4]$, **1**. The crystal structure of complex **1** has been determined by X-ray crystallography. Additionally, the biological properties of complex **1** including its binding to CT DNA investigated by UV spectroscopy and viscosity measurements, its ability to displace ethidium bromide (EB) as a means to investigate the existence of a potential intercalation to CT DNA in competition to the classical DNA-intercalator EB studied by fluorescence spectroscopy, and its affinity 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 result in lower or enhanced biological properties of the original drug, or new paths for drug transportation – investigated by fluorescence spectroscopy, have been evaluated and compared to those of free niflumic acid.

2. Experimental

2.1. Materials – instrumentation – physical measurements

Niflumic acid, $MnCl_2{\cdot}4H_2O$, 2,2′-bipyridylamine (=bipyam), 1,10-phenanthroline (=phen), 2,2′-bipyridine (=bipy), KOH, trisodium citrate, NaCl, CT DNA, BSA, HSA and EB 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.

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 1.88, 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 $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [26–28].

Infrared (IR) spectra (400–4000 cm^{-1}) were recorded on a Nicolet FT-IR 6700 spectrometer with samples prepared as KBr pellets. 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 on a magnetic susceptibility balance of Sherwood Scientific (Cambridge, UK). C, H and N elemental analysis were performed on a Perkin–Elmer 240B elemental analyzer. Molar conductivity measurements were carried out in 1 mM DMSO solution of the complexes 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 $[Mn(nif)_2(MeOH)_4]$, **1**

A methanolic solution (10 mL) containing niflumic acid (0.4 mmol, 112 mg) and KOH (0.4 mmol, 22 mg) was stirred for 1 h. The solution was added dropwise to a methanolic solution (10 mL) of $MnCl_2{\cdot}4H_2O$ (0.2 mmol, 40 mg). Colorless crystals of $[Mn(nif)_2(MeOH)_4]$ **1** suitable for X-ray structure determination were collected after a few days. Yield: 105 mg, 70%. *Anal.* Calc. for $[Mn(nif)_2(MeOH)_4]$ ($C_{30}H_{32}F_6MnN_4O_8$) (MW = 745.54): C, 48.33; H, 4.33; N, 7.52. Found: C, 47.69; H, 4.12; N, 7.25%. IR (KBr pellet): ν_{max}/cm^{-1} $\nu_{asym}(CO_2)$: 1606 (very strong (vs)); $\nu_{sym}(CO_2)$: 1389 (vs); $\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2)$: 217 cm^{-1}; UV–Vis: λ/nm ($\varepsilon/M^{-1}\ cm^{-1}$) as nujol mull: 327, 296; in DMSO: 331 (sh) (4500), 295 (22 500). μ_{eff} = 5.95 BM at room temperature. The complex is soluble in DMF and DMSO (Λ_M = 7 mho $cm^2\ mol^{-1}$, in 1 mM DMSO solution).

The addition of an N,N′-donor ligand such as bipy (0.2 mmol, 31 mg), phen (0.2 mmol, 36 mg) or bipyam (0.2 mmol, 34 mg) to the reaction solution has resulted in the isolation of crystalline product of complex **1**, and no coordination of the N,N′-donor was observed.

2.3. X-ray structure determination

Single-crystal X-ray diffraction data were collected at room temperature on an Agilent Technologies SuperNova Dual with an Atlas detector using mirror-monochromatized Mo Kα radiation (λ = 0.71073 Å). The data were processed using CRYSALIS PRO [33]. Structure was solved by direct methods implemented in SIR97 [34] and refined by a full-matrix least-squares procedure based on F^2 with SHELXL-97 [35]. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were readily located in difference Fourier maps and were subsequently treated as riding atoms in geometrically idealized positions with $U_{iso}(H) = kU_{eq}(C, N)$, where k = 1.5 for NH and methyl groups, which were permitted to rotate but not to tilt, and 1.2 for all other H atoms. Hydrogen atoms bonded to methanol O3 and O4 atoms were refined using DFIX instruction. The $-CF_3$ group is disordered over two positions in ratio 0.59:0.41. Crystallographic data are listed in Table 1.

2.4. Albumin binding studies

Protein binding studies have been performed by tryptophan fluorescence quenching experiments using bovine (BSA, 3 μM) or human serum albumin (HSA, 3 μM) in buffer (containing 15 mM trisodium citrate and 150 mM NaCl at pH 7.0). The quenching of the emission intensity of BSA or HSA tryptophan residues at 343 nm or 351 nm, respectively, was monitored using Hnif and complex **1** as quenchers with increasing concentration (up to

Table 1
Crystallographic data for complex **1**.

	Complex **1**
Formula	$C_{30}H_{32}F_6MnN_4O_8$
Fw	745.54
T (K)	293(2)
Crystal system	orthorhombic
Space group	*Pbca*
a (Å)	16.1718(3)
b (Å)	9.8618(2)
c (Å)	21.0311(4)
α (°)	90.00
β (°)	90.00
γ (°)	90.00
Volume (Å^3)	3354.10(11)
Z	4
D(calc) (Mg m^{-3})	1.476
Abs. coef., μ (mm^{-1})	0.481
GOF on F^2	1.046
R_1=	0.0364[a]
wR_2=	0.0907

[a] 3023 reflections with $I > 2\sigma(I)$.