Carbohydrate Research 346 (2011) 2886–2895

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

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

Carbohydrate-conjugate heterobimetallic complexes: synthesis, DNA binding studies, artificial nuclease activity and in vitro cytotoxicity

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ARTICLE INFO

Article history:
Received 6 August 2011
Received in revised form 1 October 2011
Accepted 7 October 2011
Available online 15 October 2011

Keyword:
N-Glycoside
Heterobimetallic
NMR interaction
DNA cleavage
Anticancer

ABSTRACT

New carbohydrate-conjugated heterobimetallic complexes $[C_{32}H_{62}N_{10}O_8NiSn_2Cl_4]Cl_2$ **(1)** and $[C_{32}H_{62}N_{10}O_8CuSn_2Cl_4]Cl_2$ **(2)** were synthesized and characterized by spectroscopic (IR, ^1H, ^{13}C, and ^{119}Sn NMR, EPR, UV–vis, ESI-MS) and analytical methods. The interaction studies of **2** with CT DNA were studied by using various biophysical techniques, which showed high binding affinity of **2** toward CT DNA. The extent of interaction was further confirmed by the interaction of **2** with the nucleotides viz.; 5′-AMP, 5′-CMP, 5′-GMP, and 5′-TMP, by absorption titration. ^1H, ^{31}P, ^{119}Sn NMR spectroscopy further validated the interaction mode of **2** with 5′-GMP. The electrophoresis pattern observed for **2** with supercoiled pBR322 DNA, exhibited significantly good nuclease activity following oxidative pathway. The preferential selectivity of **2** toward the major groove was observed on interaction of **2** with pBR322 DNA, in the presence of standard groove binders viz.; DAPI and methyl green. Additionally, in vitro antitumor activity of **2** was evaluated on a panel of human cancer cell lines, exhibiting remarkable cytotoxicity activity against Colo205 (colon) and MCF7 (breast) cell lines with GI_{50} values <10 μg/mL.

1. Introduction

In medicinal chemistry and chemical biology research, the identification of compounds with novel and defined biological functions is of high importance. The discovery of cisplatin in 1965 heralded the development of metallopharmaceuticals and founded a revolution in cancer therapy.[1–4] An interesting group of chemotherapeutic agents used in cancer therapy comprises molecules that interact with DNA. The mechanism of cytotoxicity of these drugs involves adduct formation with DNA or site specific cleavage of DNA strand, alkylation of DNA; thereby interfering with replication and transcription machinery.[5,6] Among the most common metal binding sites to DNA are the heteroatoms of nucleoside bases that form strong complexes with transition metal ions.[7] Recently, Reedijk and co-workers[8–10] have reported copper(II)–platinum(II) complexes where complexation with copper has been used as a targeting element to direct a nuclease moiety to specific DNA sites. Previously, it has been well demonstrated that the DNA cleavage activity of copper complexes could be largely affected by the ligand structure.[11,12]

Metals—in particular, transition metals—offer potential advantages over the more common organic-based drugs, including a wide range of coordination numbers and geometries, accessible redox states, tune-ability' of the thermodynamics and kinetics of ligand substitution, and a wide structural diversity. Platinum-based drugs result in serious side effects (including nephrotoxicity, gastrointestinal toxicity, neurotoxicity, and ototoxicity) and acquired drug resistance which has limited the clinical applications of these drugs.[13,14] In contrast 3d transition metal ions are biocompatible (copper is widely distributed in biological system and it has been demonstrated that copper accumulates in tumor due to selective permeability of cell membranes) which has opened up new research vistas for developing non-platinum based cancer chemotherapeutics, in particular, copper-based anticancer agents.[15,16] Heterobimetallic complexes are combination bifunctional agents containing two or more active metal entities which exhibit preferential intrinsic DNA interaction.[8–10,17,18] Sn(IV) ions possess a hard Lewis acid nature, neutralize the negative charge of the phosphate moiety of the DNA backbone and thus brings conformational changes in DNA.[19,20] Previous studies of Cu(II) and Cu(II)–Sn(IV) complexes have shown various interesting results against various cancer cell lines (HeLa, T47D, HT29).[21–24] It has been demonstrated that these complexes induce apoptosis through mitochondrial pathway. The heterobimetallic complexes enhance the chemotherapeutic action many-fold as they provide a dual mode of binding at the target site and also exhibit novelty in preferential selectivity inside the cell viz.; Cu(II) ions specifically bind to N7 of the nucleobase. On the other hand, Sn(IV) complexes prefer to bind to the oxygen atom of the phosphate of the polyanionic structure of DNA.

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0008-6215/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2011.10.010

Metal complexes containing N-glycosides derived from polyamines and D-glucosamine have shown thereby, effective antitumor activities.[25] Amino sugars namely, 2-amino-2-deoxyaldoses such as D-glucosamine are biologically interesting molecules with several donor atoms capable of binding 3d transition metal ions viz.; Co(III), Ni(II), Cu(II), and Zn(II). D-Glucosamine was reported to inhibit proliferation of cancer cells by Baek et al.[26] Appending carbohydrate moiety to drug candidates to form new drugs and/or pro-drugs, offers great potential in increasing the solubility of the molecule and minimizing the toxicity, and enhancing the stability.[27] A potential benefit of this approach leads freely available pendant form of carbohydrate to interact with transport and metabolic pathways in the body. Piperazine is a water soluble cyclic diazine with rigid preorganized cyclohexane conformation. The hexaatomic piperazine ring may exhibit a boat or a chair conformation, however the thermodynamic stability of chair form is 17.2 kJ mol^{-1} more compared with boat form.[28] The boat conformation is stabilized when the piperazine ring coordinates one metal ion through both the nitrogen atoms forming chelate complex. However, the structural character of piperazine causes some rigidity.[29–31]

In this paper, we report the synthesis of carbohydrate-conjugated heterobimetallic complexes. Complex **1** was synthesized for structural elucidation only. The interaction studies of **2** with CT DNA and nucleotides were carried out by employing various biophysical techniques. The in vitro cytotoxicity of **2** was tested against a panel of 20 human carcinoma cell lines of different histological origins.

2. Results and discussion

2.1. Synthesis and characterization

The heterobimetallic complexes $[C_{32}H_{62}N_{10}O_8NiSn_2Cl_4]Cl_2$ (**1**) and $[C_{32}H_{62}N_{10}O_8NiSn_2Cl_4]Cl_2$ (**2**), containing ethylenediamine linked N-glycoside, bis(piperazine-1,4-dicarbaldehyde)M(II)chloride (M = Ni and Cu), and $(CH_3)_2SnCl_2$ in a 2:1:2 stoichiometry, respectively, were synthesized by three step synthetic route (Scheme 1). The progress of the reaction and the purity of the products were monitored by thin layer chromatography at each step and final compounds obtained were HPLC purified. Both the complexes are soluble in H_2O and DMSO. The molar conductance values (Λ_M) of **1** and **2** in H_2O (150–200 Ω^{-1} cm^2 mol^{-1}) indicate their 1:2 electrolytic nature. On the basis of spectral studies, square planar geometry of the central metal ions viz.; Ni(II) in **1** and Cu(II) in **2** has been proposed, while the Sn(IV) atoms were present in hexacoordinated environment, which was proposed on the basis of ^{119}Sn NMR. An X-ray powder diffraction study of the microcrystalline **2** was performed to obtain further evidence about the structure of the complex. The binding studies of **2** with CT DNA were carried out by employing absorption, emission spectroscopic titrations, and cyclic voltammetry.

2.1.1. IR spectroscopy

The IR spectra of the complexes **1** and **2** exhibited a characteristic broad envelop at around 3400 cm^{-1} due to the symmetric and anti symmetric vibrations of –OH and $–NH_2$ groups of the sugar residue. A moderate peak in the region 1637–1632 cm^{-1} was assigned to δ(N–H). The broadening results from the coordination of tin metal ions to the hydroxyl groups. The characteristic bands at 1657–1658 cm^{-1} were assigned to Schiff base ν(C=N). The strong bands appearing between 1446 and 1451 cm^{-1} were attributed to δ(OCH, CH_2, CCH)[32,33] while the bands at 1389–1411 cm^{-1} were assigned to ν(C–N).[34] The far IR region exhibited characteristic bands at 618–622 cm^{-1}, 339–336 cm^{-1}, and 472–479 cm^{-1} which were attributed to ν(Sn–O), ν(Sn–N), and ν(M–N) vibrations, where M = Cu(II) and Ni(II), respectively, ascertain the chelation of tin to the sugar moiety via oxygen atom and central metal ions to the nitrogen atom of piperazine-1,4-carbaldehyde.. The ν(Sn–Cl) was also revealed by the presence of medium intensity bands at ~290 cm^{-1}.

2.1.2. NMR spectroscopy

The ^{1}H, ^{13}C, and ^{119}Sn NMR spectra of the complex **1** were recorded in D_2O. The ^{1}H NMR spectrum of **1** revealed signal at 0.88 ppm attributed to the $–CH_3$ protons of the organotin moiety. A series of multiplets in the range of 2.70–3.80 ppm were attributed to the skeletal protons of the N-glycoside moiety, piperazine ring, and the alkyl spacer.[31,35] Due to the saccharide-typical strongly coupled system, the individual sugar resonances could not be assigned. The peak at 4.71 ppm is assigned to NH of the amine. The characteristic aldimine proton peak appeared at 7.95 ppm.[36]

The ^{13}C NMR spectrum of **1** displayed signals at 11.52–11.23 ppm ascribed to the four methyl group carbons linked to the tin(IV). The signals in the range of 46.29–36.42 ppm were observed for the $–CH_2$ carbons of piperazine-1,4-carbaldehyde and ethylenediamine. The distinct peaks of the carbon atoms of D-glucosamine appeared in the range of 78.21–56.30 ppm[37] with downfield peaks corresponding to the anomeric carbons of the sugar moiety that is, (C(1)) and (C(1′)). Other carbons were observed toward the upfield side in the order (C(5)), (C(5′)), (C(3)), (C(3′)), (C(6)), (C(6′)), (C(4)), (C(4′)), (C(2)), (C(2′)) which is well supported by the literature.[38,37] In addition to these carbon signals, the characteristic aldimine carbon was observed at 163.65 ppm.

It is known that ^{119}Sn chemical shift δ is sensitive toward the coordination sphere around the tin atom. ^{119}Sn NMR spectrum of **1** showed a single peak for two tin metal atoms at −271.79 ppm due to the same environment around the tin centers, which is in good agreement with the octahedral geometry.[39]

2.1.3. EPR spectroscopy

The liquid state X-band EPR spectrum of **2** in DMSO acquired at LNT is anisotropic exhibiting two peaks $g_{\parallel} = 2.22$, $g_{\perp} = 2.07$ and $g_e = 2.0023$ (Fig. 1). Since $g_{\parallel} > g_{\perp} \sim g_e$ is indicative of a $\{d_{x^2-y^2}\}^1$ or $\{d_{z^2}\}^1$ Cu(II) ion, this pattern is consistent with Cu(II) ion in a square planar geometry. The trend $g_{\parallel} > g_{\perp} > 2$ revealed that the unpaired electron is present in the $d_{x^2-y^2}$ orbital. For a Cu(II) complex, $g_{\parallel}$ is a parameter sensitive enough to indicate covalence. For a covalent complex, $g_{\parallel} < 2.3$ and for an ionic environment, $g_{\parallel} = 2.3$ or more. In the present complex **2**, $g_{\parallel} < 2.3$ indicating an appreciable metal–ligand covalent character.[40,41]

2.1.4. XRPD measurements

Since a suitable sized single crystal was not obtained despite several efforts, we have performed the X-ray powder diffraction pattern (XRPD) to further elucidate the structure of the complex. The result suggested the microcrystalline nature of **2**. The lattice parameter a = 7.330 Å, b = 7.330 Å, and c = 7.864 Å and the values for $\alpha = \beta = 90°$, $\gamma = 120°$ show hexagonal crystal lattice and the lattice type is P. The 2θ ranges from 20 to 79.18. The diffractogram obtained for **2**, is depicted in Figure 2.

2.2. Binding studies

2.2.1. Absorption spectral titrations

DNA binding studies were carried out only with copper complex **2**, and its analogous nickel complex **1** was synthesized only for NMR studies. When the complex **2** was titrated with CT DNA, interesting spectral changes in the ligand-field band were observed, the absorption spectral traces of **2** with increasing concentration of CT