Journal of Inorganic Biochemistry 103 (2009) 128–134

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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

Palladium(II) binding to N(7) of acyclovir: DNA interaction and herpes simplex virus (HSV-1) inhibitory activity

J. Gómez-Segura [a,*], S. Caballero [b], V. Moreno [a,*], M.J. Prieto [b], A. Bosch [b,*]

[a] Departament de Química Inorgànica, Universitat de Barcelona, Diagonal 647, E-08028 Barcelona, Spain
[b] Departament de Microbiologia, Universitat de Barcelona, Diagonal 645, E-08028 Barcelona, Spain

ARTICLE INFO

Article history:
Received 14 May 2008
Received in revised form 25 September 2008
Accepted 25 September 2008
Available online 1 November 2008

Keywords:
2′-Deoxyguanosine analogue
Acyclovir-antiherpes agent
Palladium(II) complex
HSV-1 inhibitory activity

ABSTRACT

Cytotoxicity and herpes simplex virus (HSV-1) inhibitory activity of acyclovir (ACV), 9-[(2-hydroxyethoxy)methyl]guanine, and the palladium(II) coordination complex *cis*-$[PdCl_2(H_2O)(N7\text{-}ACV)] \cdot ACV \cdot xH_2O$ have been tested in African green monkey kidney (Vero line) epithelial cell cultures. The N(7) position of ACV represents the preferred binding site to afford a pseudo-chelate N7/O6 Pd(II) complex involving H-bonds with the *cis* H_2O molecule. The Pd(II)–ACV complex has been structurally characterized by FTIR and ^1H NMR spectroscopy techniques, chemical composition was measured by elemental analysis, and the thermoanalytical study was performed by TG/DTA. The recognition of secondary ACV molecules by the Pd(II) derivative promotes cooperatively potent HSV-1 inhibitory activity which, in turn, strongly depends on concentration conditions. At the optimal concentration of 10 μM, this complex exhibits antiviral efficiency *in vitro*, approximately hundred-fold (*ca.* $1.87 \log_{10}$) more effective in herpes-infected cells when compared with that of the parent ACV molecules. The molecular-level observation of noticeable modifications caused by the complex on the morphology of the plasmid pBR322 DNA was monitored by AFM, whose mutual interaction evolves to eventually afford DNA condensates upon increasing the period of incubation.

© 2008 Elsevier Inc. All rights reserved.

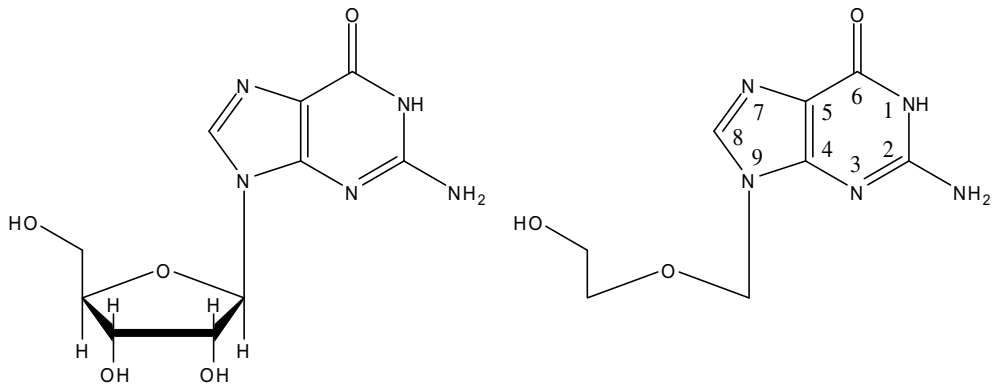
1. Introduction

Acyclic nucleoside analogues can function as representative antiviral agents with potential applications in the field of virology and more generally biomedicine. The archetypal acyclovir molecule (ACV), 9-[(2-hydroxyethoxy)methyl]guanine, is a synthetic nucleoside analogue derived from guanine that further contains an acyclic side chain (Scheme 1). Importantly, it acts as a potent antiherpic drug that exhibits highly specific inhibitory activity against the proliferation of herpes simplex viruses (HSV), especially HSV type-1 (HSV-1) and type-2 (HSV-2) [1]. *In vitro* studies have proved that the pharmacologically active form of ACV results from its enzymatic phosphorylation to acyclovir triphosphate (ACV·TP) [2–4], which preferentially inhibits the viral DNA polymerase. The low affinity of the α-DNA polymerase for ACV·TP results from both the minimal uptake and phosphorylation of ACV into uninfected cells, thereby leading to reduced cytotoxic effects [5,6]. On the one hand the ACV·TP molecules compete with deoxyguanosine triphosphate for the viral DNA polymerase, but on the other hand the DNA replication results inhibited caused by further incorporation of ACV·TP into the growing chains of the viral DNA molecule [2–4]. The replacement of the carbohydrate moiety of deoxyguanosine (ribofuranosyl ring) by the modified acyclic deoxyribose substituent of ACV, namely, the attached 2-hydroxyethoxymethyl chain, accounts for the eventual termination of the DNA synthesis.

Interestingly, the mechanism of action and toxic side effects of purely organic drugs can be modulated in the presence of metals. These are essentially required by the majority of enzymes for their catalytic activity in virus-infected and uninfected cells [7–9]. For instance, the role of Zn(II) in the biological activity of some DNA polymerases and the concomitant activation by other metal ions as Mg(II), Mn(II) or Co(II). No less important is the role of metal-targeted drugs with different coordination behaviour. These allow, in view of structural changes and properties, modified biological activity by affecting the metal environment of enzymes. On this basis, extensive studies have been focused on metal complexes of biologically active ligands. Investigations into the metal coordination properties of DNA nucleobases and derivatives are thus of current interest. Of all the recognized binding sites on DNA, N(7)-coordination of the purine nucleobases guanine and adenine is preferred by most metal species [10–13]. Besides, hybrid drugs of Pt(II) or Pd(II) derivatives can simultaneously exhibit differing antiviral and/or anticancer activity with respect to their constituents based on synthetic nucleoside analogues [14,15]. Within the variety of transition metal coordination compounds of ACV, particularly with the divalent metal species of Co,

* Corresponding authors. Tel.: +34 93 402 1914; fax: +34 93 490 7725.
E-mail address: virtudes.moreno@qi.ub.es (V. Moreno).

0162-0134/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2008.09.018



Scheme 1. Representation of the molecular structure of deoxyguanosine (left) and acyclovir (right). The carbohydrate moiety of deoxyguanosine is replaced by the modified, acyclic deoxyribose substituent (2-hydroxyethoxymethyl chain) of the nucleoside analogue acyclovir.

Ni, Cu, Cd, Hg and Pt [16–24], uniquely those containing Cu(II), Pt(II) or Zn(II) exhibit antiherpes simplex efficiency *in vitro,* the latter resulting by far more active against HSV-1 viruses [14,15,25,26]. By comparison with unplatinated ACV, the Pt(II) complex of ACV *cis*-$[PtCl(NH_3)_2(ACV)]NO_3$ prepared in the seminal work by Coluccia et al. maintained the antiviral activity, though exhibiting minor efficacy on a molar basis, i.e. the 50% inhibitory concentrations (ID_{50}) [14]. Likewise, both ACV and the prototype Pt(II) complex $[Pt(NH_3)_2(ACV)_2](NO_3)_2$ evidenced similar inhibitory activity against HSV-1 strain KOS. Regardless, the ACV derivative obtained by ligand substitution from the precursor $[Pt(NH_3)_2Cl_2]$ complex precludes the normal cells from cytotoxicity. Hitherto, García-Raso et al. have reported unprecedented recognition of free ACV molecules represented by the Ni(II) and Co(II) complexes of ACV [24], and herein we report the preparation and structural characterization of the powerful antiherpic Pd(II) complex of ACV, namely *cis*-$[PdCl_2(H_2O)(N7\text{-}ACV)]$ ACV $2H_2O$. To the outstanding antiviral properties, a new prospective hybrid drug stems from the observation of enhanced interaction affinity with DNA as is preliminary conducted by AFM studies.

2. Experimental

2.1. Material and methods

The Pd(II) coordination complex was prepared using the reagents and solvents as received from Sigma/Aldrich and no further purification was carried out. ACV was synthesized by the method of Matsumoto et al. [27].

Elemental analysis (C, H and N) were performed on a Carlo Erba EA1108 micro analyzer at the Serveis Científico-Tècnics of the University Rovira i Virgili of Tarragona.

Mid- and far-IR spectra were obtained in solid state on a FTIR Nicolet-Impact 400 spectrometer using KBr and polyethylene pellets, respectively.

TG–DTG/DTA data were measured in the 30–700 °C temperature range at a heating rate of 5 °C min^{-1} in a dry air flow of 70 cm^3 (STP) min^{-1}, using a Mettler Toledo TGA/SDTA851e microbalance equipped with a 34-position sample robot. Typically, the solids (2–4 mg) were put in 70 μl-alumina crucibles without dilution.

^1H NMR spectra were recorded on an Unity-300 spectrometer. ^1H chemical shifts were measured relative to tetramethylsilane (TMS) and dimethyl sulfoxide-d_6 (DMSO-d_6) as the solvent.

Mass conductivity measurements were directly monitored on a Crison 525 conductimeter at room temperature. A 10^{-3} M solution of the metal complex was made up in DMSO and standardized by analytical grade KCl (0.01 M) in doubly deionized water (<1 μS cm^{-1}). Cell constant was fixed at 0.530 cm^{-1} as determined by the KCl standard solution in accordance with the reference mass conductivity value at 25 °C [28].

All atomic force microscopy (AFM) observations were made with a Nanoscope III Multimode AFM (Digital Instrumentals, Santa Barbara, CA). Nano-crystalline Si cantilevers of 125-nm length with a spring constant of 50 N/m average ended with conical-shaped Si probe tips of 10-nm apical radius and cone angle of 35° were utilized. High-resolution topographic AFM images were performed in air at room temperature on different specimen areas of 2 × 2 μm operating in intermittent contact mode at a rate of 1–3 Hz.

2.2. Synthesis of the complex

Acyclovir (1 mmol) was added to an aqueous solution of $K_2[PdCl_4]$ (0.5 mmol, 50 mL). The resulting suspension was stirred for 24 h at room temperature and, by this time, a crude, yellow precipitate was formed. The solid was filtered off and air dried to be subsequently added to dimethylformamide (10 mL). The mixture was warmed under stirring up to 50 °C and hot filtered. The brownish precipitate was removed by filtration and thoroughly washed in water, methanol and ethyl ether. Yield: 0.2913 g, 76%. $[C_{16}Cl_2H_{28}N_{10}O_9Pd]$ requires: C, 28.2; H, 4.1; N, 20.5. Found: C, 28.6; H, 3.9; N, 20.3. Conductivity (10^{-3} M in DMSO, 25 °C): 2.74 Λ_M/Ω^{-1} cm^2 mol^{-1}.

2.3. Antiviral activity assays

The inhibitory effects of the Pd(II) complex and unpalladated ACV on the HSV replication were evaluated by the inhibition of virus-induced cytopathogenicity in an African green monkey kidney (Vero line) epithelial cells. Vero cell cultures were used to propagate and assay a reference strain of HSV-1. Vero cells (HSV-1; ATCC VR-539, American Type Culture Collection, Manassas, VA, USA) were seeded at ~280,000–300,000 cells/mL in 96-well microtiter plates. The cell monolayers thus obtained were infected with HSV-1 viruses in serial 10-fold dilutions and incubated for 1 h at 37 °C in a humidified CO_2-controlled atmosphere. The serial dilutions were performed in 5-mL tubes using phosphate buffered saline solution (PBS) as the diluent. Tenfold dilutions from 10^{-1} to 10^{-6} were made up (0.9 mL of diluent with 0.1 mL of the previous dilution). The compounds under study were dissolved in doubly distilled water containing 2% of DMSO to a concentration of 100 μM. Subsequent to virus attachment, infected cells were washed and cultured with 150-μl medium of Eagle's minimal essential medium (MEM) supplemented with 2% heat-inactivated

Download English Version:

https://daneshyari.com/en/article/1316881

Download Persian Version:

https://daneshyari.com/article/1316881

Daneshyari.com