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Energetics, conformation, and recognition of DNA duplexes containing a major adduct of an anticancer azolato-bridged dinuclear Pt^{II} complex

Jarmila Mlcouskova [a,b], Jaroslav Malina [b], Vojtech Novohradsky [a,b], Jana Kasparkova [a,b], Seiji Komeda [c], Viktor Brabec [b,*]

[a] Department of Biophysics, Faculty of Science, Palacky University, 17. listopadu 12, CZ-77146 Olomouc, Czech Republic
[b] Institute of Biophysics, Academy of Sciences of the Czech Republic, Kralovopolska 135, 61265 Brno, Czech Republic
[c] Faculty of Pharmaceutical Sciences, Suzuka University of Medical Science, Suzuka 513-8670, Japan

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ABSTRACT

Background: The design of anticancer metallodrugs is currently focused on platinum complexes which form on DNA major adducts that cannot readily be removed by DNA repair systems. Hence, antitumor azolato-bridged dinuclear Pt^{II} complexes, such as $[\{cis\text{-}Pt(NH_3)_2\}_2(\mu\text{-}OH)(\mu\text{-pyrazolate})]^{2+}$ (AMPZ), have been designed and synthesized. These complexes exhibit markedly higher toxic effects in tumor cell lines than mononuclear conventional cisplatin.
Methods: Biophysical and biochemical aspects of the alterations induced in short DNA duplexes uniquely and site-specifically modified by the major DNA adduct of AMPZ, namely 1,2-GG intrastrand cross-links, were examined. Attention was also paid to conformational distortions induced in DNA by the adducts of AMPZ and cisplatin, associated alterations in the thermodynamic stability of the duplexes, and recognition of these adducts by high-mobility-group (HMG) domain proteins.
Results: Chemical probing of DNA conformation, DNA bending studies and translesion synthesis by DNA polymerase across the platinum adduct revealed that the distortion induced in DNA by the major adduct of AMPZ was significantly less pronounced than that induced by similar cross-links from cisplatin. Concomitantly, the cross-link from AMPZ reduced the thermodynamic stability of the modified duplex considerably less. In addition, HMGB1 protein recognizes major DNA adducts of AMPZ markedly less than those of cisplatin.
General significance: The experimental evidence demonstrates why the major DNA adducts of the new anticancer azolato-bridged dinuclear Pt^{II} complexes are poor substrates for DNA repair observed in a previously published report. The relative resistance to DNA repair explains why these platinum complexes show major pharmacological advantages over cisplatin in tumor cells.

1. Introduction

Di- and trinuclear platinum complexes have attracted much interest as alternative drugs to cisplatin [*cis*-diamminedichloridoplatinum(II)] and its analogs in cancer chemotherapy [1–3]. Azolato-bridged dinuclear Pt^{II} complexes constitute a group of anticancer metallodrugs which exhibit a very promising in vitro cytotoxicity [4,5]. These bifunctional complexes appear to be novel anticancer agents with a mechanism of action different from those of mononuclear cisplatin and its analogs used in the clinic. There is a large body of evidence indicating that the cytotoxicity of platinum complexes correlates with their ability to bind DNA [6–8]. Azolato-bridged dinuclear Pt^{II} complexes interact with DNA, and the DNA binding mode of these Pt^{II} complexes is distinctly different from that of cisplatin and other polynuclear Pt^{II} complexes [4,9,10].

A Pt^{II} drug candidate belonging to the class of antitumor azolato-bridged dinuclear Pt^{II} complexes is $[\{cis\text{-}\{Pt(NH_3)_2\}_2(\mu\text{-}OH)(\mu\text{-}$ pyrazolate$)\}]^{2+}$ (AMPZ) (Fig. 1). It contains two platinum centers that are each capable of binding DNA monofunctionally after loss of the bridging hydroxide [9]. Global platination of highly polymeric natural DNA with a random nucleotide sequence with AMPZ gives rise to major 1,2-GG intrastrand cross-links (CLs) similar to cisplatin [11]. Unlike cisplatin, AMPZ induces in highly polymeric DNA only small

Abbreviations: AMPZ, $[\{cis\text{-}\{Pt(NH_3)_2\}_2(\mu\text{-}OH)(\mu\text{-pyrazolate})\}]^{2+}$; cisplatin, [*cis*-diamminedichloridoplatinum(II)]; bp, base pair; CL, cross-link; CT, calf thymus; DSC, differential scanning calorimetry; EMSA, electrophoretic mobility shift assay; FAAS, flameless atomic absorption spectrometry; DMS, dimethyl sulfate; dNTP, deoxyribonucleotide triphosphate; HPLC, high-pressure liquid chromatography; HMG, high mobility group; HMGB1a, domain A of full length HMGB1 protein; HMGB1b, domain B of full length HMGB1 protein; LD, linear dichroism; T_m, melting temperature; NER, nucleotide excision repair; PAA, polyacrylamide; Polη, DNA polymerase η; r_b, the number of molecules of the platinum complex bound per nucleotide residue; SDS, sodium dodecyl sulfate; XPA, xeroderma pigmentosum group A

* Corresponding author. Tel.: +420 541517148; fax: +420 541240499.
E-mail addresses: sandrock@centrum.cz (J. Mlcouskova), malina@ibp.cz (J. Malina), novohradskyv@gmail.com (V. Novohradsky), jana@ibp.cz (J. Kasparkova), komedas@suzuka-u.ac.jp (S. Komeda), brabec@ibp.cz (V. Brabec).

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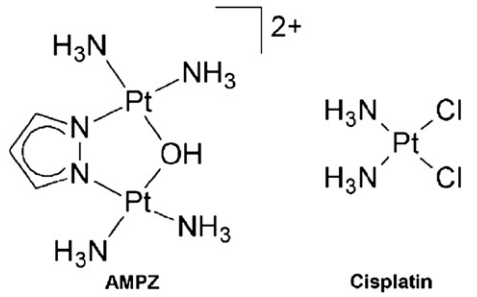

Fig. 1. Structure of cisplatin and azolato-bridged dinuclear Pt^{II} complex.

conformational distortions. In general, after DNA is coordinatively modified by platinum-based anticancer drugs, cellular repair systems recognize this damage and struggle to correct it, which may give rise to the toxic effects of these metallodrugs in tumor cells. This is why DNA repair is considered to play a significant role in modulating the cytotoxicity of platinum drugs [12–14]. The small conformational alterations induced in DNA by AMPZ represent markedly weaker structural motifs recognizable by DNA repair systems compared to distortions induced by cisplatin. This assumption has been experimentally confirmed. We demonstrated in our recent work [11] that DNA adducts of AMPZ can escape repair mechanisms more easily than those of cisplatin, which may potentiate antitumor effects of these new metallodrugs in cancer cells.

The results of previous work [5,11,15] are consistent with the view that major DNA adducts of AMPZ distort the double helical structure of DNA much less than those of conventional cisplatin. Herein, we have studied some important biophysical and biochemical aspects of the alterations induced in short synthetic DNA duplexes uniquely and site-specifically modified by the major DNA adduct of AMPZ, namely 1,2-GG intrastrand CLs. We compare these biophysical and biochemical properties with those obtained under identical conditions for the same adduct of cisplatin. Particular attention is paid to details of conformational distortions induced by the adducts of AMPZ and cisplatin, associated alterations in the thermodynamic stability of the duplexes containing these adducts, and recognition of these adducts by high-mobility-group (HMG) domain protein, that is, the important factors that modulate the antitumor effects of platinum drugs already used in the clinic.

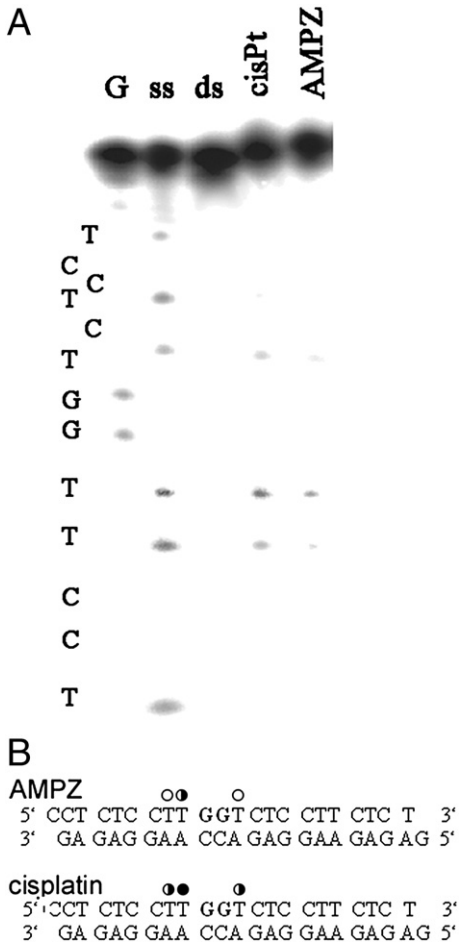

Fig. 2. Chemical probing of DNA conformation. A. Piperidine-induced specific-strand cleavage at $KMnO_4$-modified bases in the 22-bp duplex (shown in Fig. 2B) unplatinated or containing a single, site-specific 1,2-GG intrastrand CL of AMPZ or cisplatin at the central GG residues in the top strand. The oligomers were 5′-end labeled on the top strand. Lanes in Fig. 2A (the top strand 5′-end labeled): G: a Maxam–Gilbert specific reaction for G residues (DMS) in the unplatinated top strand; ss: the unplatinated single strand; ds: the unplatinated duplex; cisPt and AMPZ: the duplex containing a unique adduct of cisplatin and AMPZ, respectively. B. Summary of the reactivity of the chemical probe with the 22-bp duplex containing a single, site-specific adduct of AMPZ and cisplatin. The platinated nucleobase is highlighted in bold. Full, half-full, and open spots designate strong, medium, and weak reactivity, respectively.

2. Materials and methods

2.1. Chemicals

Cisplatin (purity was ≥99.9% based on elemental and ICP trace analysis) was obtained from Sigma (Prague, Czech Republic). The dinuclear azole-bridged Pt^{II} complex AMPZ was synthesized according to published procedures [4,9]. Stock solutions of the platinum complexes [5×10^{-4} M in $NaClO_4$ (10 mM)] were stored in the dark at 277 K. The concentrations of platinum in the stock solutions were determined by flameless atomic absorption spectrometry (FAAS). Calf thymus (CT) DNA (42% G + C, mean molecular mass ca. 20 000 kDa) was prepared and characterized as described previously [16,17]. T4 DNA ligase, Klenow fragment of DNA polymerase I, and T4 polynucleotide kinase were purchased from New England Biolabs (Beverly, MA). DNA polymerase η (Polη) was from EnzyMax, LLC (Lexington). The synthetic oligodeoxyribonucleotides were purchased from VBC-GENOMICS (Vienna, Austria) or DNA Technology (Aarhus, Denmark). The purity of the oligonucleotides was verified by either high-pressure liquid chromatography (HPLC) or gel electrophoresis. In the present work, the molar concentrations of the single-stranded oligonucleotides or duplexes are related to the oligomers (not to the monomer content) or double-stranded molecules, respectively. Molar extinction coefficients for the single-stranded oligonucleotides (related to the 12–23-mer strands) were determined by phosphate analysis [18]. The formation of 1:1 complexes between the top strands unmodified or containing the intrastrand adduct and bottom strands of the duplexes was verified by recording isothermal UV absorbance mixing curves at 298 K [19]. The N-terminal His6-tagged xeroderma pigmentosum group A (XPA) protein was obtained by expressing the plasmid DNA pET15b/XPA template [20] in RTS 500 *Escherichia coli* HY (Roche) and purified on Ni^{2+}–NTA agarose and by hydroxyapatite chromatography [21]. The plasmid DNA pET15b/XPA was kindly provided by Richard D. Wood. Acrylamide, bis(acrylamide), NaCN, dithiothreitol, and urea were from Merck KgaA (Darmstadt, Germany). Dimethyl sulfate (DMS) and $KMnO_4$ were from Sigma (Prague, Czech Republic). Deoxyribonucleotide triphosphates (dNTPs) were from Roche Diagnostics, GmbH (Mannheim, Germany). Sodium dodecyl sulfate (SDS) was from Serva (Heidelberg, Germany). Expression and purification of domains A (residues 1–84 [22] and B (residues 85–180 [22]) (HMGB1a and HMGB1b, respectively) of recombinant rat full-length HMGB1 protein (HMG = high mobility group) were carried out as