Journal of Inorganic Biochemistry 166 (2017) 135–140

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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

Aza-bridged bisphenanthrolinyl Pt(II) complexes: Efficient stabilization and topological selectivity on telomeric G-quadruplexes

Lei He [a,1], Zhenyu Meng [a,1], Yi-qun Xie [b,1], Xiang Chen [a], Tianhu Li [a], Fangwei Shao [a,*]

[a] Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 637371, Singapore
[b] Department of Breast Surgery, Shanghai Huangpu Center Hospital, Shanghai 200002, China

ARTICLE INFO

Article history:
Received 1 June 2016
Received in revised form 30 September 2016
Accepted 3 November 2016
Available online 09 November 2016

Keywords:
Pt complex
G-quadruplex
RNA
Telomere
Folding topology
Anti-cancer activity

ABSTRACT

Two platinum complexes with an aza-bridged bis(1,10-phenanthrolin-2-yl)amine (bpa) were synthesized. The two phenanthrolines in bpa entered a flat plane prior to binding of nucleic acids, which bestowed on the two Pt complexes a significantly high stabilizing ability on both DNA and RNA G-quadruplexes. Further extending alkyl tail from aromatic coordination core enabled the complexes to distinguish GQ sequence based upon the topological folding structures and enhanced the selectivity of the complex against duplex DNA. This study paved the way to develop Pt complexes as GQ stabilizers for specific folding topology and the applications to disease and/or personalized anticancer medicine/therapy.

1. Introduction

Nucleic acids with guanine rich sequences have a propensity to adopt tetra-stranded intra- and intermolecular quadruplex structures that are stabilized by a stack of planar aromatic guanine quartets (G-tetrad), in which four guanines are held together by noncanonical Hoogsteen hydrogen bonding [1–5]. Formation and stability of the whole G-quadruplex structure usually exhibited monovalent cation-dependent manner: $Li^+ < Na^+ < K^+$ [6–9]. These G-rich sequences can adopt different topologies with distinct strand orientation, including parallel, antiparallel and parallel/antiparallel hybrids [10–17]. G-quadruplexes (GQ) involved in a number of key cellular processes. The well-known example is the human telomeric DNA, which is consists of tandem arrays of simple G-rich (TTAGGG) repeats over several hundred bases [18–20]. It has been shown that if this single-stranded telomeric DNA folded into a quadruplex, the activity of telomerase will be inhibited and thus impede cell proliferation [21,22]. Recent studies found that telomeric RNA (*TERRA*), which is transcribed from the C-rich strand of telomeric DNA, was involved in chromatin regulation and remodeling, as well as telomerase function [23–25]. Besides, many oncogene promoters possess G-quadruplex folding sequences, such as *c-myc*, *bcl-2* and *c-kit*, form rich GQ topology in vitro and in vivo [26]. The significant biological roles of G-quadruplex suggested GQ, as a unique family of noncanonical secondary structure, have high potentials as anticancer drug targets for chemotherapeutics.

Up until now, considerable studies focused on the stabilization of G-quadruplexes by using small molecules, such as porphyrin-based complexes and heteroaromatic organic compounds [27–32]. These compounds with planar aromatic motifs can stack with the multiple layers of G-tetrads at the core of G-quadruplex. At the same time the extensive size and shape of the large aromatic conjugates were also used to distinguish duplex DNA and achieve specific binding to GQs. Besides, sequence and cation dependence of folding polymorphism make it possible to target a specific G-rich sequence by interactions between binding molecules and unique loop arrangements. Consequentially, a GQ stabilizer with binding preference to a specific G-quadruplex topological structure can reach the goal of disease specific and/or personalized medicines.

Metal complexes have been reported to strongly and selectively interact with quadruplex DNA [33–38]. As G-quadruplex binders, metal complexes display several advantageous over their organic counterparts. Metal complex with planar heteroaromatic ligand provided strong π stacking with G-tetrad so that efficiently stabilized G-quadruplexes. Besides, both the coordination geometry of metal center, the ligand size and alkyl tails extended from aromatic moieties were easily tuned to obtain topological recognition of GQ targets. Moreover, the electropositive metal center endowed its interaction with cation cavity at the center of G-quadruplexes. Although these metal complexes have shown excellent binding affinity and stabilization on DNA G-

* Corresponding author.
E-mail address: fwshao@ntu.edu.sg (F. Shao).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jinorgbio.2016.11.011
0162-0134/

quadruplexes, none of them has been demonstrated as RNA G-quadruplex stabilizer until now.

To date, a lot of platinum complexes have been reported as G-quadruplex stabilizers. The square shape of di-ligand coordination on Pt^{2+} provides the necessary size and shape of aromatic area for good π-π stacking with G-quartets [39–43]. Metal complexes coordinated with phenanthroline and its derivatives have been widely studied as GQ binders and stabilizers due to its excellent size matched with G-quartet. Herein, we designed and synthesized two platinum complexes with bis(1,10-phenanthrolin-2-yl)amine (bpa) ligand. Two 1,10-phenanthroline (phen) were connected via an aza-bridge and form a planar configuration under acidic condition. Upon coordination, two $[Pt(bpa)]^{2+}$ complexes can enter a rigidly flat geometry and bestow strong π stacking with G quartets to enhance GQ stability. Both Pt complexes exhibited significantly higher stabilization effect towards both DNA and RNA G-quadruplexes than duplex DNA.

2. Materials and methods

All the chemicals and RNA sequence were purchased from Sigma-Aldrich. All DNA sequences were purchased from Sangon (Shanghai, China). The synthetic route of *N*-methyl-*N*-(1,10-phenanthrolin-2-yl)-1,10-phenanthrolin-2-amine (**L1**) and *N*-(1,10-phenanthrolin-2-yl)-*N*-(2-(piperidin-1-yl)ethyl)-1,10-phenanthrolin-2-amine (**L2**) were described in supporting information. Milli-Q water was used in all physical measurement experiments. ^{195}Pt NMR spectra were recorded on a Bruker AVIII 400 MHz NMR spectrometer. Chemical shift was referenced externally to K_2PtCl_4 in D_2O (δ −1628 ppm) for ^{195}Pt NMR spectra.

2.1. Synthesis of [Pt(bpa)]Cl_2 complexes

[Pt(L1)]Cl_2 (**1**). Pt(dmso)$_2Cl_2$ (11.0 mg) and **L1** (10 mg) were brought to reflux in a mixture of methanol and water with the ratio of 1:1. After 5 h, the mixture was cool to room temperature and yielded orange precipitates. The crudes were collected by filtration and were washed with methanol for three times to provide the product in 78% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.98 (d, J = 4.5 Hz, H2,2′, 2H), 9.35–9.25 (m, H4,4′,7,7′, 4H), 8.69 (d, J = 9.3 Hz, H8,8′, 2H), 8.53–8.33 (m, *H*3,3′,5,5′,6,6′, 6H), 4.53 (s, 3H). ^{195}Pt NMR (86 MHz, DMSO-d_6): δ −2272. ESI-MS calcd for $[M - Cl]^+$ 617.08, $[M - 2Cl]^{2+}$ 291.06. Found $[M - Cl]^+$ 616.96, $[M - 2Cl]^{2+}$ 290.96.

[Pt(L2)]Cl_2 (**2**). Pt(dmso)$_2Cl_2$ (11.0 mg) and **L2** (12.5 mg) were reflux in methanol. After 5 h, the mixture was cool to room temperature and yielded yellow precipitates. The crudes were collected by filtration and were washed with methanol for three times to provide the product in 85% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.05 (d, J = 4.7 Hz, H2,2′, 2H), 9.38 (d, J = 9.2 Hz, H7,7′, 2H), 9.33 (d, J = 8.2 Hz, H4,4′, 2H), 8.90 (d, J = 9.2 Hz, H8,8′, 2H), 8.58 (d, J = 8.6 Hz, H6,6′, 2H), 8.52–8.45 (m, J = 7.6 Hz, *H*3,3′,5,5′, 4H), 5.49 (t, J = 7.3 Hz, H1,1′, 2H), 3.86 (t, J = 7.3 Hz, H9,9′, 2H), 3.66 (d, J = 11.3 Hz, H10,10′, 2H), 3.15 (s, H10″,10‴, 2H), 1.90 (m, H11″,11‴,12,12′ 4H), 1.80 (d, J = 11.7 Hz, H11, 1H), 1.52–1.37 (m, H11′, 1H). ESI-MS calcd for $[M - Cl]^+$ 714.17, $[M - 2Cl]^{2+}$ 339.60. Found $[M - Cl]^+$ 715.04, $[M - 2Cl]^{2+}$ 339.19.

2.2. Thermal melting assay

Thermal melting of G-quadruplexes and double stranded DNA were obtained by CD melting. 100 μL of *c-myc* or HT21 (3 μM in 10 mM lithium cacodylate buffer, pH = 7.4, without KCl), 100 μL of telomeric RNA (6 μM in 10 mM potassium phosphate buffer, pH = 7.0, 15 mM KCl) and 100 μL of ds26 (5 μM in 5 mM potassium phosphate buffer, pH = 7.4) were annealed by heating at 95 °C for 5 min and gradually cooling to room temperature over couple hours. Pt complexes were added to DNA solution to yield a final concentration of 3 μM or 5 μM (only for ds26). Thermal melting was monitored at 260 nm for *c-myc*, 295 nm for HT21, 263 nm for telomeric RNA and 250 nm for ds26, at the heating rate of 0.5 °C/min from 20 °C to 95 °C. Melting temperatures were analyzed by Origin 8.0 (OriginLab Corp.). Standard deviation over three repeat experiments were used as error bars.

2.3. UV–vis absorption titration

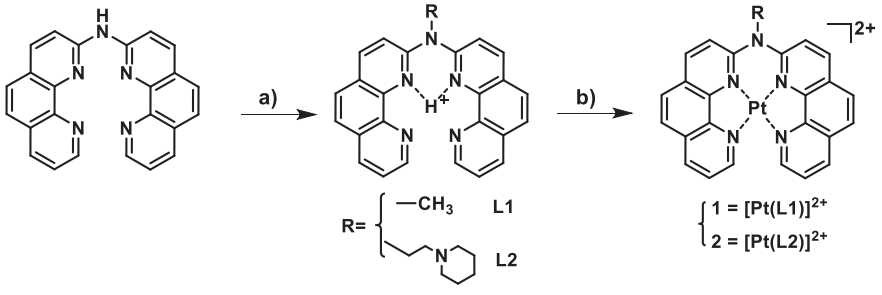
For UV titration experiment, 100 μL of Pt complex solution (20 μM in 100 mM KCl, 10 mM Tris HCl, pH 7.4) was titrated with 1 μL of DNA stock (100 μM in 100 mM KCl, 10 mM Tris HCl, pH 7.4) at 25 °C. Binding association constant K (M^{-1}) was calculated with the literature method [44]. The results were got from average of three replicates.

2.4. CD spectrum and titration

CD spectra were recorded on a Jasco J-1500 spectropolarimeter using a 1 cm path length cuvette. DNA stock solutions were diluted to 3 μM with 10 mM lithium cacodylate buffer (pH 7.4) with 1 mM KCl for *c-myc* and 10 mM KCl for HT21. 12-nt RNA stock solution was diluted to 6 μM with 10 mM potassium phosphate buffer (pH = 7.0) with 15 mM KCl, respectively. CD spectra were recorded from 220 to 320 nm at a scan rate of 200 nm/min after each titration of complex to DNA solutions. All CD spectra were baseline-corrected and each curve represented of five averaged scans taken at 25 °C. Final analysis of the data was carried out using Origin 8.0 (OriginLab Corp.).

2.5. Molecular docking of Pt complexes to GQ topological structures

1 and **2** were first drawn by Gaussian view followed by an optimization step (B3LYP/6-31g* for C, H, N; SDD for Pt) using Gaussian 09 to optimize the coordination structures of two Pt complexes [45]. In docking simulation, GOLD Suite v5.4 (CCDC Software Limited) is used to dock the optimized structures of two complexes, **1** and **2**, into the basket (PDB ID: 2mcc) and hybrid G-quadruplex structures (PDB ID: 2mb3), respectively [46]. Pt cation was set as a dummy atom for docking simulation. The scoring function was calculated by CHEMPLP. In each simulation, all the torsional angles within 15 Å to the binding site, i.e. the center of the external G-quartet layer, was free to rotate. Subsequently genetic algorithm was employed to find the maximum fitness value



Scheme 1. Synthesis of the platinum complexes.