Colloids and Surfaces B: Biointerfaces 156 (2017) 320–329

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Protocols

Biophysical and biological studies of some polymer grafted metallo-intercalators

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ARTICLE INFO

Article history:
Received 8 December 2016
Received in revised form 8 May 2017
Accepted 13 May 2017
Available online 15 May 2017

Keywords:
Polymeric drug conjugates
Polymer metal complexes
DNA binding study
Anticancer activity

ABSTRACT

Two water-soluble polymer-copper(II) complexes, $[Cu(ip)_2(BPEI)](ClO_4)_2{\cdot}H_2O$ (Complex 1) and $[Cu(dppz)_2BPEI](ClO_4)_2{\cdot}H_2O$ (Complex 2) with different degree of coordination have been synthesized and characterized. The interaction between the prepared complexes and CT—DNA has been assessed by various physico-chemical methods The spectroscopic and the cyclic voltammetry studies have revealed that both the complexes interact with CT—DNA through intercalation binding mode. Among the two complexes, Complex 2 has higher binding affinity with CT—DNA. The antiproliferative activity of the complexes has been examined on human breast cancer cells, MDA—MB—231, adopting various techniques. The results indicate that both the polymer-copper(II) complexes are effective against the breast cancer cell line and the order of the activity is consistent with the DNA-binding ability.

1. Introduction

Over the years, many metal complexes and organic molecules have been reported as anticancer agents. However, their poor water solubility, metabolic instability and dose-dependant toxicity have restricted them to be used in clinical trails [1–3]. Therefore, many research groups have started to design anticancer drugs without the above mentioned disadvantages. Grafting metal complexes and organic molecules into the polymer make them into good candidate for chemotherapy [4,5]. The unique features of polymeric drug conjugates mainly depend on the choice of the polymer backbone [6–8]. Especially, the cationic polymer, branched polyethyleneimine (BPEI) and its modified forms have been used for gene delivery [9], drug delivery [10], and cell imaging [11]. A recent report indicates that, BPEI increases the cellular uptake of iridium metal complexes in cancer cells [12]. The advantage of buffering capcity of pH of PEI is that it increases the antiproliferative activity in chemotherapeutic drug [9]. Based on the above facts, many reports suggest that BPEI anchored metal complexes have shown promising therapeutic properties than that of ordinary metal complexes [13–15].

Copper is an endogenous metal and essential for angiogenesis, which makes copper based chemotherapeutic agents very popular over the years [16–19]. Further, the redox nature and biocompatibility of copper makes it's complexes as potential drug for a variety of diseases. [20–23]. As DNA is the fundamental molecule for cell division, protein synthesis and development of the organism, it serves as a supreme target for many drugs. Moreover, the structure and the composition of DNA offers many target cites for metal complexes. Therefore, studies on the interactions of copper complexes with DNA have been an active field in chemotherapeutic drug desinging. This motivates us to study the importance of polymer anchored copper complexes and their interactions with CT-DNA and proteins [24–26].

In general, the metal complexes containing polypyridyl ligands like ip, dpq and dppz (ip = imidazo[4,5-f]1,10-phenanthroline, dpq = dipyridio[3,2-d;2′,3′-f]quinoxaline, dppz = dipyrido[3,2-a:2′,3′-c]phenazine) are better metallo-intercalators [27–29]. Further, these complexes induce π-stacking interactions with the π- electron clouds of the DNA base pairs which leads to the unwinding of DNA double helix and provide interesting photophysical properties [30,31]. Our earlier studies show that BPEI anchored copper(II) complexes containing phenanthroline/bipyridine and amino acid mixed ligands displayed electrostatic interaction with DNA/RNA [32–34]. However, BPEI containing metallo-intercalator

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http://dx.doi.org/10.1016/j.colsurfb.2017.05.037
0927-7765/

$[Cu(dpq)_2BPEI](ClO_4)_2{\cdot}2H_2O$ shows dual interactions such as electrostatic and π-π stacking with DNA/RNA and its binding affinity affects cell viability of the non metastatic MCF-7 cancer cells [35]. Continuing, our efforts in understanding the efficacy of the polymer anchored copper(II) complexes, here, we report the binding ability of BPEI grafted copper complexes containing polypyridyl ligands, (ip and dppz) with DNA. Further, the anti-cancer activity of these synthesized complexes have been tested on MDA-MB-231 cells.

2. Experimental section

2.1. Material

Calf thymus DNA (CT-DNA), branched polyethyleneimine (BPEI) (Mw ca. 25,000) and copper(II) perchlorate hexahydrate were purchased from Sigma-Aldrich, and ammonium acetate and formaldehyde were obtained from Merck, India. Glacial acetic acid from Qualigens Fine Chemical, India. Ortho phenylenediamine and Tris-HCl were received from Loba Chemie, and sodium chloride (NaCl) from SISCO Research laboratories, India. The ligands (ip and dppz) and the precursor complexes, $[Cu(ip)_2(H_2O)](ClO_4)_2$ and $[Cu(dppz)_2(H_2O)](ClO_4)_2$ were prepared by literature methods [36,37]. The carbon, hydrogen and nitrogen contents of the samples were determined at SAIF, CERI, Karaikudi, India. Copper analysis was done by a reported procedure [38]. Infra-red spectra were recorded on FT-IR JASCO 460 PLUS spectrophotometer with samples prepared as KBr pellets. EPR spectra were recorded on JEOL-FA200 EPR spectrometer.

Human breast cancer cell line, MDA-MB-231 (ATCC, USA.) were cultured as a monolayer in Dulbecco's Modified Eagles Medium (Biochrom AG, Berlin, Germany), supplemented with 10% fetal bovine serum (Sigma-Aldrich, USA), with 100 μg/mL of streptomycin (Himedia, Mumbai, India) as antibiotics, at 37 °C, in a humidified atmosphere containing 5% CO_2, in a CO_2 incubator (Heraeus, Hanau, Germany).

2.2. Absorption spectral titration

The DNA binding experiments were performed at 25.0 ± 0.2 °C. A solution of calf thymus DNA in the buffer gave a ratio of UV absorbance at 260–280 nm of ~1.8–1.9:1, indicating that the CT-DNA was sufficiently free of protein [39]. The concentration of CT-DNA in base pairs was determined by UV absorbance at 260 nm by taking the molar extinction coefficient value 13200 $M^{-1}cm^{-1}$ for CT-DNA at 260 nm [40,41]. Absorption spectral titration experiments were performed by maintaining constant concentration of the polymer complexes with varying the CT-DNA concentration on a Shimadzu UV–vis spectrophotometer. An equal amount of CT-DNA was added to both the complex and the reference solution to eliminate the absorbance of CT-DNA itself.

2.3. Competitive binding studies

Emission spectra were recorded on a JASCO FP 770 spectrofluorimeter. For fluorescence quenching experiments, CT-DNA was pre-treated with ethidium bromide (EB) for 30 min. Solutions of complexes were then added to this mixture and their effect on the emission intensity was measured. The samples were excited at 450 nm and emission was observed between 500 and 700 nm. The spectra were analysed according to the classical Stern-Volmer equation [42],

$$I_0/I = 1 + K_{sv}r, \quad (1)$$

where I_0 and I are the fluorescence intensities in the absence and the presence of complex, respectively. K_{sv} is a linear-Stern-Volmer quenching constant dependent on the ratio of r_{bE} (the ratio of the bound concentration of ethidium bromide to the concentration of DNA). r is the ratio of the total concentration of complexes to that of CT-DNA.

2.4. Cyclic voltammetry

All cyclic voltammetry experiments were performed in a single compartment cell with a three electrode configuration on a Princeton EG and G-PARC model potentiostat. Glassy carbon was the working electrode, saturated calomel as the reference electrode and the platinum wire was auxiliary electrode. The supporting electrolyte was 50 mM NaCl/5 mM Tris- HCl buffer at pH 7.1. The electrode surfaces were freshly polished with alumina powder and then sonicated in ethanol and distilled water for 1 min prior to each experiment and the electrode was rinsed with doubly distilled water thoroughly between each polishing step. Before the experiments, solutions were deoxygenated by purging with nitrogen gas for 15 min prior to the measurements.

2.5. Synthesis of polymer grafted copper (ii) complexes

The precursor complex, $[Cu(ip)_2(H_2O)](ClO_4)_2$ or $[Cu(dppz)_2(H_2O)](ClO_4)_2$ and BPEI, were taken in the 2.5:1 molar ratio in methanol and the mixture was heated between 60 and 65 °C for 10 h in a water bath. The obtained dark green coloured solution was dialyzed approximately at15 °C against cold distilled water for 5–6 days, till the absence of colour in the liquid where the dialyzer is placed. Afterwards the solvent was evaporated by a rotary evaporator under reduced pressure. The dark greenish filmy polymer complex obtained was pulverized and dried. Polymer complexes with different degrees of copper(II) complex units grafted onto BPEI were prepared by varying the amount of the precursor copper(II) complex, change the reaction time etc. These complexes are very stable in solution. When we occasionally kept the solutions of the complexes in dialysis bags we never observed the presence of any free quantity of copper complex ion or copper ion in the solution outside the dialysis bag indicating that the polymer-copper(II) complexes are very stable.

2.6. Synthesis of different degree of coordination (x) of polymer-copper (ii) complexes

To a solution of 0.15 g of BPEI in 20 mL of methanol, 0.1 g, 0.2 g and 0.3 g of the precursor complexes, $[Cu(ip)_2(H_2O)](ClO_4)_2$/$[Cu(dppz)_2(H_2O)](ClO_4)_2$, in 40 mL of methanol was added. This mixture was heated between 60 – 65 °C for 6 h, 10 h and 12 h respectively. The ratio of the 1°, 2° and 3° amines of the monomeric unit of the BPEI are 1:2:1 [43]. From this ratio, the average monomeric unit of the BPEI consider as (~NH—CH_2—CH_2~). The 'x' represents the degree of coordination, which is the number of moles of copper(II) complex units per mole of the repeating unit (amine groups) of polymeric ligand. If the entire repeating units (amine groups) in the polymer are coordinated to copper, the value of x is 1. It can be calculated either from carbon content [43,44] or copper content [38]. The degrees of coordination (x) thus obtained for the complex 1 are 0.084, 0.231 and 0.487 for complex 2 are 0.102, 0.119 and 0.144.

2.7. Cell culture

MDA-MB-231 cells were cultured in Dulbecco's Modified Eagles Medium, supplemented with 10% fetal calf serum (FCS), 100 μg/mL streptomycin, and 100 μg/mL penicillin as antibiotics, at 37 °C, in a humidified 5% CO_2 in CO_2 incubator.