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ctDNA binding affinity and *in vitro* antitumor activity of three Keggin type polyoxotungestates

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ABSTRACT

The ctDNA-binding properties and *in vitro* antitumor activity of three water soluble Keggin type polyoxometalates (POMs): $K_6H[CoW_{11}O_{39}CpZr]\cdot nH_2O$, $K_6H[CoW_{11}O_{39}CpTi]\cdot nH_2O$ and $K_7H_2[CoW_{11}O_{39}CpFe]\cdot nH_2O$ (abbreviated as CoWCpZr, CoWCpTi and CoWCpFe, respectively) were investigated using UV–Vis absorption spectroscopy, fluorescence spectrophotometry, cyclic voltammetry and MTT assay. The results of UV–Vis, fluorescence and cyclic voltammetry rule out intercalating binding mode and propose the groove or outside stacking binding of these POMs with ctDNA. The values of $1.30 \times 10^4\ M^{-1}$, $1.15 \times 10^4\ M^{-1}$ and $3.10 \times 10^3\ M^{-1}$ were obtained for binding constant of CoWCpZr, CoWCpTi and CoWCpFe to ctDNA, respectively. The redox potential of POMs shift to more negative values in the presence of ctDNA which can be related to domination of electrostatic interaction in this system. The antitumor activity tests of these polyoxometalates (POMs) were carried out on two types of human cancer cells, MCF-7 and HEK-293 by MTT method. The results show the higher antitumor activity of CoWCpFe respect to two other that is related to its highest penetrating effectiveness for MCF-7 cells. Therefore, the antitumor activity of these POMs depends not only on their affinity to ctDNA but also strongly on their penetration ability to the cell membrane.

1. Introduction

Despite tremendous efforts in the development of new drugs and therapeutic procedures, cancerous diseases are still difficult to treat and the development of effective anticancer medicines remains one of the main goals of modern medicinal chemistry research. As cancer cells are highly proliferative tissues, one of the most promising biological targets to decrease tumor cell growth is the DNA [1]. DNA is the primary intracellular target for anticancer drugs; interaction between small molecules and DNA can cause damage in cancer cells, blocking the division and resulting in cell death [2,3]. Among the agents directly interacting with DNA, polyoxometalates (POMs) complexes have gained considerable attention based on their DNA-interaction properties [4–6]. POMs are early transition metal–oxygen anionic clusters [7–10]. Among numerous remarkable properties, they have been reported as having promising antibacterial, antiviral (particularly anti HIV), antitumor and anticancer activities, which may open the way toward new, and cheap therapeutic strategies for various human diseases [4–6]. During the last decades, it has been recognized that the biological activity of many polyoxoanions relate to their size, shape and charge density [11,12]. The first drug of this group that reached the clinical trial stage was $(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]$ (HPA-23), which was rejected from antitumor drugs list due to its marked toxicity and insufficient antiviral activity [13,14]. POMs are large (in the nanometer size range) and highly negatively charged species. These two factors certainly do not facilitate their penetration into cell. One might therefore conclude that the observed activities results from the interaction of the POMs with the cell surface. Some studies however indicate that under certain circumstances, POMs can cross the barrier and penetrate inside a cell [13,15–17]. In the last few years, Liu, Pope and their co-workers have investigated the cytotoxic properties of many heteropolyoxotungstates against cancer cell lines *in vitro*. For instance, the antitumor activity of a $[CoW_{11}O_{39}(CpTi)]^{7-}$ *in vitro* was investigated and its inhibitory action on three types of human cancer cells, SSMC-7721, HL-60 and HLC *in vivo* was studied [4,17]. This complex exhibited the highest antitumor activity *in vitro* among the cyclopentadienyltitanium substituted heteropolytungstate such as $(CpTi)GeW_{11}$, $(CpTi)GaW_{11}$, $(CpTi)BW_{11}$, α-$(CpTi)_3SiW_9$ and β-$(CpTi)_3SiW_9$. In order to assess the reason of antitumor activities by the POMs, the interaction of β-$K_4H_3[SiW_9O_{37}(CpTi)_3]$ with

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DNA was investigated by agarose gel electrophoresis. The results indicated that antitumor action mechanism of the β-$(CpTi)_3SiW_9$ is attributable to its ability to cleave DNA or to altering the conformation of DNA molecules. Recently, anticancer properties of tungstosilicicpolyoxometalate were also carried out by the methyl thiazolyltetrazolium (MTT) method [6]. The results showed that incorporation of neodymium into the polyoxometalate helps to significantly increase the biological activity of this polyoxometalate. It has also been reported that POMs containing a $[CpTi]^{3+}$ (abbreviation of $[Ti(\eta^5\text{-}C_5H_5)]$) group have significant antitumor activity [4]. Yamase and co-workers studied the antitumor activity [18] and enzymatic binding of heptamolybdate to DNA by electrophoresis. It was shown that the heptamolybdate binds unspecifically to single and double stranded DNA with little recognizable modifications of the DNA structure [19].

Despite of the above mentioned studies, there is no report in literature corresponding to the molecular interaction of POMs with DNA using spectroscopic and voltametric methods. Therefore, in the present work, the interactions of $K_6H[CoW_{11}O_{39}CpZr]\cdot nH_2O$, $K_6H[CoW_{11}O_{39}CpTi]\cdot nH_2O$ and $K_7H_2[CoW_{11}O_{39}CpFe]\cdot nH_2O$ (abbreviated as CoWCpZr, CoWCpTi and CoWCpFe, respectively) with ctDNA were investigated by using various techniques such as UV–Vis, fluorescence and cyclic voltammetry (CV). Moreover, the antitumor properties of these POMs were evaluated using MTT assay technique. The results were interpreted on the basis of POMs structure.

2. Experimental

2.1. Reagents and materials

Dicyclopentadienyltitaniumdichloride (Cp_2TiCl_2), dicyclopentadienylzirconiumdichloride (Cp_2ZrCl_2), dicyclopentadienyliron (Cp_2Fe), sodium tungstate dihydrate ($Na_2WO_4.2H_2O$), cobalt(II) acetate ($Co(OAc)_2\cdot 4H_2O$), acetylacetone ($C_5H_8O_2$), acetic acid ($C_2H_4O_2$) and ethanol (C_2H_5OH), were obtained from Merck company. The ctDNA and ethidium bromide (EB) were purchased from Sigma. All of the reagents and solvents were analytical grade and used without further purification. The stock solution of ctDNA was prepared by dissolving ctDNA in phosphate buffer solution at 4 °C under intense stirring for more than 24 h to get a homogeneous solution. The concentration of ctDNA solution was determined by spectrophotometry ($\varepsilon_{260} = 6600\ M^{-1}\ cm^{-1}$). 10 mM phosphate buffer solution (PBS), pH 6.2 was used in all experiments. Also, MCF-7 (breast cancer) and HEK-293 (Human Embryonic Kidney) cancer cell lines, RPMI-1640 without phenol red (Bio-Idea, Iran), penicillin–streptomycin (Sigma), Fetal bovine serum (FBS) (Sigma), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) (Bio-Idea, Iran), DMSO (Merck) and phosphate buffer saline (CMG company, Iran) were used for determination of POMs cytotoxicity.

2.2. Procedures

CoWCpTi was synthesized and purified according to the previously reported method [4] (see supporting information section). Synthesis methods for CoWCpZr and CoWCpFe are similar to CoWCpTi analog as refer to Ref. [4] (the detailed synthesis procedures for these POMs are given in the supporting information section). The synthesized POMs were characterized by using FT-IR (Jasco 6300 FT-IR spectrometer) and UV–Vis (Jasco 670 UV–Vis spectrophotometer). These informations are given in Table 1 and Figs. 1 and 2 of the supporting information section. The concentrations of K, W, Co, Zr, Ti and Fe were detrmined by a X-ray fluorescence (XRF) (Bruker, S4 pioneer, Germany) spectroscopy, the C and H contents were determined using a CHNS elemental analyzer (CHNS-932, Leco, USA). Water was estimated by thermogravimetry (TG, Mettler TA4000) (see the supporting information section).

2.3. DNA binding experiments

The absorption titration experiments were carried out by sequential addition of specified volume of ctDNA stock solution into a 1 cm path length cuvette containing POM solution (500 μL). The absorption spectra were recorded after each addition of ctDNA solution from 500 to 800 nm. The intrinsic binding constant (K_b) of POMs with ctDNA was determined by using the following equation (Eq. (1))

$$[ctDNA]_T/(\varepsilon_{[app]}-\varepsilon_f) = [ctDNA]_T/(\varepsilon_b-\varepsilon_f) + 1/K_b(\varepsilon_b-\varepsilon_f) \quad (1)$$

where ε_{app}, ε_f and ε_b correspond to A_{obs}/[POM], the extinction coefficient of free and fully bound POM, respectively. A plot of $[ctDNA]_T/(\varepsilon_{app}-\varepsilon_f)$ vs. $[ctDNA]_T$, where $[ctDNA]_T$ is the total concentration of ctDNA in the base pairs unit, gives K_b as the ratio of the slope to intercept [20–24].

The percent of hypochromisity (%H) was calculated according to the following equation:

$$\%H = (A_0 - A_F)/A_0 \times 100 \quad (2)$$

where A_0 is the absorption of POM in the absence of ctDNA, A_F is the ultimate (final) value of absorption when no change is observed due to the further addition of ctDNA to POM solution. The CV were recorded using a μ-Autolab type III (Eco Chemie, Utrecht, The Netherlands) controlled by a microcomputer with GPES 4.9 software. A three-electrode cell including glassy carbon electrode as the working electrode, a glassy carbon rod as the counter electrode and Ag/AgCl (3.0 M KCl) as the reference electrode were used. The working electrode was carefully polished prior to each series of experiments with alumina (0.05 μM) on a polishing cloth. The cyclic voltammograms of POM solution (450 μM) in the absence and presence of various amounts of ctDNA were measured at 25 °C. In order to eliminate the oxidation and reduction currents of ctDNA, the cyclic voltammograms of ctDNA solutions in the absence of POM were also determined as a control experiment.

The fluorescence spectra were recorded at 25 °C on a Shimadzu RF-5000 spectrophotometer coupled to a data recorder, using the application included in the RF – 5000 digital station software. Excitation and emission slit widths were set as 5 and 10 nm, respectively. In competition binding experiments, the concentration of EB and ctDNA were 75 μM and 180 μM, respectively, while POMs concentration varied from 0 to 86.5 μM. The 515 nm was chosen as the excitation wavelength and the emission spectra were recorded from 520 to 700 nm. The efficiency of quenching of a luminophore species by a quencher species follows the Stern–Volmer equation (Eq. (3)) [25,26].

$$F_0/F = 1 + K_{SV}[Q] \quad (3)$$

where F_0 and F are the fluorescence intensities in the absence and presence of the quencher, respectively. [Q] is the quencher concentration and K_{SV} is the Stern–Volmer quenching constant [27].

In order to further investigate the binding mode between POMs and ctDNA, fluorescence Scatchard plots for the binding of EB to ctDNA in the presence of various concentrations of POMs were obtained on the basis of previous published method [28–30]. The Schatchad equation for binding of small molecules to ctDNA is defined as follow (Eq. (4)):

$$r/C_f = K_a(n - r) \quad (4)$$

where r stands for $(CB/[ctDNA]_T)$ (CB: is the concentration of bound luminophore), C_f is the concentration of free luminophore, n is the number of binding sites per base pair of ctDNA and K_a is the