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Supporting effect of polyethylenimine on hexarhenium hydroxo cluster complex for cellular imaging applications

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ABSTRACT

The present work introduces an insight into pH- and concentration-dependent binding of branched polyethylenimine (PEI) with hexarhenium hydroxo cluster complex $[\{Re_6S_8\}(OH)_6]^{4-}$ aimed at enhancing the stability, luminescence intensity and cellular uptake of the cluster complex in aqueous solutions at pH 7.4. Luminescence and conductivity measurements revealed pH 7.4 and 1:1 of PEI:cluster molar ratio as specific conditions ensuring multiple electrostatic interactions of protonated amino groups of PEI with the tetra-anionic cluster units. These interactions resulted in a significant supporting effect of PEI on stability and luminescence of the cluster complex, while no effect of the cluster complex on the acid-base and aggregation properties of PEI was detected from dynamic light scattering and ^{1}H NMR spectroscopy measurements. The cluster luminescence remained without degradation for at least two weeks due to the supporting effect of PEI, which can facilitate the application of the cluster complex in bioimaging. The cellular uptake of $[\{Re_6S_8\}(OH)_6]^{4-}$ and $[\{Re_6S_8\}(OH)_6]^{4-}$@PEI was investigated. Confocal microscopic studies showed that the pristine cluster complex $[\{Re_6S_8\}(OH)_6]^{4-}$ did not enter cells (the human larynx carcinoma cell line (Hep-2)), whereas the supramolecular assembly $[\{Re_6S_8\}(OH)_6]^{4-}$@PEI internalized into the cells.

1. Introduction

Binding of luminescent molecules or complexes with water soluble polymers have attracted a great deal of attention as a tool to enhance the solubility, hydrophilicity, biocompatibility and other functional properties [1–8]. In turn, in recent years, luminescent hexanuclear cluster complexes have been recognized as a promising platform for various biological and medical applications [9–21], although their biocompatibility and cellular uptake behavior are still poorly studied.

Hexarhenium cluster complex $[\{Re_6S_8\}(OH)_6]^{4-}$ is of particular impact in bioanalysis due to its high luminescence and kinetic inertness of the cluster core $\{Re_6S_8\}$, which is the prerequisite of its application in cellular imaging. Unfortunately, two significant shortcomings restrict their use in cellular imaging. The first is the decrease in the luminescence of $[\{Re_6S_8\}(OH)_6]^{4-}$ in neutral aqueous solutions due to protonation of the apical hydroxyls and time-induced photodegradation. The second shortcoming is the insignificant cellular uptake of the anionic cluster complexes. The modification of hexanuclear cluster complexes by amphiphilic organic ligands, encapsulation of the complexes into dendrimers, silica nanoparticles or metal-organic mesoporous framework were previously reported as convenient synthetic routes to gain in cellular uptake behavior [9,10,17–20]. Non-covalent or supramolecular route of the cluster modification is a promising alternate to the above mentioned synthetic procedures.

Multicharged polyelectrolytes have got wide applications in the development of nanosized systems for drug delivery and sensing [22–26]. Moreover, they are well recognized as a promising basis for nonviral polymeric vectors facilitating cellular uptake behavior for both molecules and nanosized particles [27]. The ability of

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polyelectrolytes to wrap around oppositely charged nanosized templates or bind small counter-ions is well documented in literature [28–35]. Both binding modes are possible in an assembly of tetra-anionic nanosized luminescent hexarhenium cluster complex [36] $[\{Re_6S_8\}(OH)_6]^{4-}$ with polyethylenimine (PEI). Indeed, formation of a supramolecular assembly of the cluster anion with the polyelectrolyte should result in mutual influence of $[\{Re_6S_8\}(OH)_6]^{4-}$ and PEI on their functional properties, which is the reason for studying such systems by different physical methods. Thus, the present work introduces specific binding of protonated PEI with nanosized cluster anions $[\{Re_6S_8\}(OH)_6]^{4-}$ resulting in improved photophysical properties and cellular uptake behavior of the metal cluster units.

2. Experimental section

2.1. Materials

Branched polyethylenimine ($MW_{average}$ = 25000) (Sigma-Aldrich), sodium chloride (Sigma-Aldrich), potassium hydroxide (Sigma-Aldrich), hydrochloric acid concentrate (Fluka), DAPI (4′,6-diamidine-2′-phenylindole dihydrochloride) from Sigma-Alddrich and Alexa Fluore-488 phalloidin from ThermoFisher Scientific were used as commercially received without further purification. $K_4[\{Re_6S_8\}(OH)_6]\cdot 8H_2O$ was synthesized and purified according to previously published procedure [36].

2.2. Cell culture

Human larynx carcinoma cell line (Hep-2) was purchased from the State Research Center of Virology and Biotechnology VECTOR and cultured in Eagle's Minimum Essential Medium (EMEM, pH = 7.4) supplemented with a 10% fetal bovine serum under a humidified atmosphere (5% CO_2 and 95% air) at 37C.

2.3. Methods

The steady-state emission spectra were recorded on a FL3-221-NIR spectrofluorometer (Horiba Jobin Yvon) at 350 nm excitation wavelength. Luminescence decay measurements were performed on the Fluorolog-3-221 spectrofluorometer with a SPEX FL-1042 phosphorimeter accessory using a xenon flash lamp as the photon source with following parameters: time per flash 50.00 ms, flash count 200, initial delay 0.05 ms, and sample window 2 ms. Excitation was performed at 350 nm, and emission was detected at 650 nm with 5 nm slit width for both excitation and emission. The steady state and time resolved luminescence measurements were carried out under oxygenated conditions. All the measurements were performed within one hour after the sample preparation.

NMR experiments were performed on a Bruker AVANCE_III-600 spectrometer equipped with the 5 mm broadband probe head.

Dynamic light scattering (DLS) measurements were carried out with a Malvern Mastersize 2000 particle analyzer at 25 °C. Experimental autocorrelation functions were analyzed with the Malvern DTS software and the second-order cumulant expansion methods. The average error was ca. 4%.

pH of solutions was controlled with Microprocessor pH meter «pH 212» (Hanna Instruments). The pH-meter was calibrated with standard aqueous buffer solutions.

Conductivity measurements were carried out on "InoLab Cond Level 1" (Weilheim, Germany) with TetraCon 325/Pt electrode.

All samples were prepared in deionized water filtered through PVDF membrane with a Syringe Filter (0.45 μm). All measurements were performed at least in triplicate at 25 °C.

Confocal Laser Scanning Microscopy of the cells after their internalization by $[\{Re_6S_8\}(OH)_6]^{4-}$ or $[\{Re_6S_8\}(OH)_6]^{4-}$@PEI was performed by using a Zeiss LSM 710/NLO confocal microscope (Carl Zeiss Inc., Jena, Germany) equipped with a laser diode (405 nm) for luminescence excitation and with an objective EC Plan-Neofluar 40x, NA = 1.30, resolution 0.15 mkm/pixel. The images were obtained and analyzed with ZEN 2009 software.

Hep-2 cells were seeded on slides (1.5×10^5 cells/slide) and incubated overnight at 37 °C under a 5% CO_2 atmosphere. The medium was then replaced with a fresh medium containing 75 μM of $[\{Re_6S_8\}(OH)_6]^{4-}$ or 19 μM of $[\{Re_6S_8\}(OH)_6]^{4-}$@PEI and incubated for 24 h. The cells incubated in the absence of cluster complex were used as a control. The cells were washed twice with phosphate-buffered saline (PBS), fixed in 4% paraformaldehyde, and washed three times with PBS. Then the cells were permeablized with 0.1% Triton X-100 in PBS (pH 7.4) for 5 min at room temperature, and washed thrice with PBS. F-actin was then stained with Alexa Fluore-488 phalloidin for 15 min at room temperature and washed thrice with PBS. Finally, coverslips were washed thrice with PBS and water, and sealed using mounting medium for fluorescence with DAPI.

2.4. MTT-assay

The effect of $[\{Re_6S_8\}(OH)_6]^{4-}$ and $[\{Re_6S_8\}(OH)_6]^{4-}$@PEI on the metabolic activity of the cells was determined using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. The Hep-2 cells were seeded into 96-well plates at 7×10^3 cells/well in a medium containing $[\{Re_6S_8\}(OH)_6]^{4-}$ or $[\{Re_6S_8\}(OH)_6]^{4-}$@PEI with concentrations from 2.7 to 600 μM and then incubated for 72 h under 5% CO_2 atmosphere. 10 μL of the MTT solution (5 mg/mL) was added to each well, and the plates were incubated for 4 h and then the formazan formed was dissolved in DMSO (150 μL). The optical density (*OD*) was measured with a plate reader Multiskan FC (Thermo scientific, USA) at a wavelength of 620 nm. The experiment was repeated three times on separate days.

Percentage of viable tumor cells was calculated by the formula: (viable cells)% = (*OD* of drug treated sample/*OD* of untreated sample) × 100.

3. Results and discussions

3.1. Mutual effects of $[\{Re_6S_8\}(OH)_6]^{4-}$ and PEI on spectral properties and conductivity

Presentation of the results should be preceded by a discussion of the main driving forces of binding and binding modes between the anionic cluster complex and PEI. Electrostatic interactions are the most common basis for binding various anions with PEI. Nevertheless, in the case of $[\{Re_6S_8\}(OH)_6]^{4-}$, possible apical OH—ligand substitution should be taken into account, since amino-groups of PEI are potentially able to substitute the hydroxo ligands. The apical ligand substitution can be monitored by luminescence measurements of $[\{Re_6S_8\}(OH)_6]^{4-}$ in different solutions since the luminescence properties of hexarhenium cluster complexes are strongly sensitive to the $\{Re_6S_8\}^{2+}$ core ligand environment.

Fig. 1 presents the spectral data for solutions of $K_4[\{Re_6S_8\}(OH)_6]\cdot 8H_2O$ in water, in aqueous solutions of PEI and in ammonia for the comparison. The results point to significant difference in spectral pattern of the cluster in PEI- and ammonia-based solutions at pH 9.2.

Fig. 1b shows emission intensity of the cluster complex at 650 nm measured at various pH. The pH of the aqueous cluster solutions was adjusted by hydrochloric acid or potassium hydroxide solution. As it was shown before [37], in an aqueous