Journal of Photochemistry and Photobiology B: Biology 140 (2014) 396–404

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Multifunctional material based on ionic transition metal complexes and gold–silica nanoparticles: Synthesis and photophysical characterization for application in imaging and therapy

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ARTICLE INFO

Article history:
Received 21 March 2014
Received in revised form 3 September 2014
Accepted 6 September 2014
Available online 16 September 2014

Keywords:
Gold–silica nanoparticles
Ionic ruthenium(II) and iridium(III) complexes
Photodynamic therapy
Cell imaging
Multifunctional nanomaterials

ABSTRACT

A new combination of luminescent ionic transition-metal complexes (**M** = Ru(II) or Ir(III)) with gold silica-based nanoparticles (**GSNPs**) gives a promising nanomaterial for application in biomedical fields. Herein we report the synthesis and the photophysical properties of Ru(II) and Ir(III) complexes doped gold core-polysiloxane shell particles prepared by microemulsion method and characterized by Transmission Electron Microscopy, Dynamic Light Scattering and UV–Vis spectroscopy. The cytotoxicity and photodynamic activity of the obtained 50 nm-diameter nanoparticles were evaluated *in vitro*, providing noteworthy results. Furthermore, their intrinsic phosphorescence allows the localization of the photosensitizing nanoparticles into the cytosol of tumor cells by fluorescence confocal microscope. These valuable features designate them as multifunctional nanoplatforms for theranostic purposes.

1. Introduction

Sumer and Gao [1] in an editorial appeared in 2008 on Nanomedicine stated: "*Theranostics was coined originally as a term to describe a treatment platform that combines a diagnostic test with targeted therapy based on the test results. Here, we define theranostic nanomedicine as an integrated nanotherapeutic system, which can diagnose, deliver targeted therapy and monitor the response to therapy*". Such statement has been followed by several papers that promote the concept and importance of theranostic agents [2]. It was emphasized that an optimal theranostic system should be constituted of (i) a sensor for the transduction of a specific signal of target, and (ii) an active species able to exert the therapeutic effect. In this framework, some researchers has proven that transition metal complexes (TMCs) may be efficient theranostic agents because of (i) they guide the bioavailability of some metal ions with specific targeting for improving diagnostics [3] (especially those with a charge-transfer excited state and long-lived emission in the visible region) and (ii) they induce local cytotoxicity for efficient therapy [4]. By a proper choice of metal center and ligands, it is possible to combine a variety of photophysical and photochemical properties almost inaccessible for a simple molecule system [5]. In fact, the properties of a coordination compound are something more than the simple sum of the properties of the ligand and metal.

Actually, Photodynamic Therapy (PDT), for its non-invasive property, has been appreciated for the treatment of neoplastic diseases and non-malignant conditions, including age-related macular degeneration and psoriasis [6].

Complexes with a high spin–orbit coupling constant could be very useful for applications in PDT; indeed, the acceleration of intersystem crossing rate from the absorbing singlet electronic excited state towards triplet states (coupled by their long-living triplet excited states) increases the probability of interaction with the molecular oxygen ground state, leading to the generation of highly reactive singlet oxygen $^1O_2$. Several TMCs containing Ru(II), Re(II), Ir(III), Pt(II) have been reported in literature and their ability to generate cytotoxic reactive oxygen species under visible light

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http://dx.doi.org/10.1016/j.jphotobiol.2014.09.005
1011-1344/

irradiation (photosensitizing effect) proven [7]. Despite their luminescence efficiency, the wide diffusion of TMCs as theranostic agents has to take into account some difficulties such as insufficient solubility, modulation of distribution, targeting, and local delivery [8]. These drawbacks may be overcame by embedding the TMCs within nanoparticles [9]. Among them, silica nanoparticles appear as particularly interesting due to their solubility in aqueous biological medium, their synthetic versatility, as well as their functionalization tunability. Various strategies [10], and in particular the microemulsion methods [11], allows the production of highly monodisperse and spherical particles with sizes ranging from 20 to 100 nm that favor the encapsulation of ionic TMCs *via* noncovalent interactions [12].

Ru(II) or Ir(III) complexes have been widely used as photosensitizers, due to their long-living triplet excited states that favor energy transfer towards dioxygen [7a,13]. Nevertheless, up to now, only few papers have reported the synthesis and photophysical characterization of nanoparticles embedding Ru(II) [14] or Ir(III) [15] complexes, used simultaneously as PDT photosensitizers, sensors and imaging active species.

In this paper we present the synthesis and photophysical characterization of new ionic Ru(II) complexes, **Ru₁** and **Ru₂**, and the photophysical properties of luminescent ionic Ir(III) complexes, **Ir₁** and **Ir₂** (Chart 1), highlighting their $^1O_2$ photosensitation ability. To exploit these interesting features, **Ru₁**, **Ru₂**, **Ir₁** and **Ir₂** have been encapsulated into gold silica nanoparticles (GSNPs) by one-pot microemulsion synthetic method. As well known, the nanoparticles size plays a critical role in their pharmacokinetics and tissue distribution; in particular, NPs with a diameter of approximately 50 nm, exhibit an enhanced rate of tumoral uptake and retention [16]. In this perspective, 50 nm GSNPs have been synthesized, morphologically and photophysically characterized, and their specific cytotoxic capability for living cells tested.

2. Materials and methods

2.1. Synthesis of the octahedral ionic Ru(II) and Ir(III) complexes

All chemicals were used as received without further purification. For the synthesis of **Ru₁** and **Ru₂** complexes, the related 2,2′-bipyridine(bpy)-derived ligands *bpy-C₁₁* and *bpy-C₁₁Si* were initially synthesized (Scheme 1).

In particular, 4,4′-dimethoxy-2,2′-bipyridine was converted into 4,4′-dihydroxy-2,2′-bipyridine (**1**) in a mixture of HBr/CH_3COOH under reflux [17], and then reacted with the corresponding bromine derivatives. The silyl bromide chains of *bpy-C₁₁Si* ligand were previously obtained from the commercially available 11-bromo-1-undecanol and *tert*-butyldimethylsilyl chloride. These ligands were used for the synthesis of **Ru₁** and **Ru₂** using a three-step process reported in the literature [18], starting from *cis*-$Ru(bpy)_2Cl_2$ hydrate as precursor (see Electronic Supplementary Information).

The structural characterization of the Ru(II) complexes was performed by elemental analysis, IR and ^1H NMR spectroscopies. The ionic character was evidenced by conductivity measurements. The values found in acetonitrile solutions (about $350\ \Omega^{-1}\ cm^2\ mol^{-1}$) were indicative of 2:1 electrolytes [19]. In the IR spectra of **Ru₁** and **Ru₂**, the characteristic intense band at $840\ cm^{-1}$ indicates the presence of PF_6^- counter ions. The ^1H NMR spectra of the complexes are consistent with the expected structures (see Electronic Supplementary Information).

The synthesis of the Ir(III) complexes (**Ir₁** and **Ir₂**) containing 2-phenylpyridine (*ppy*) as cyclometallating ligands was reported elsewhere [20].

2.2. Synthesis of gold-core nanoparticles with Ru(II) and Ir(III) complexes doped silica shell

Chemicals. 4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glicol (Triton X-100), n-hexanol, cyclohexane, hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4·3H_2O$), sodium 2-mercaptoethanesulfonate (MES), sodium borohydride ($NaBH_4$), tetraethoxysilane (TEOS) and (3-aminopropyl)triethoxysilane (APTES) were purchased from Aldrich. Ammonia solution (25 wt%) (NH_4OH) was purchased from Chimie Plus. N-(3-triethoxysilyl)propylsuccinic anhydride (94%) was purchased from ABCR. Only Milli-Q water ($\rho > 18\ M\Omega$) was used for the preparation of the aqueous solution.

Synthesis. GSNPs embedding **Ru₁**, **Ru₂**, **Ir₁**, **Ir₂** metal complexes (**Ru₁@GSNP**, **Ru₂@GSNP**, **Ir₁@GSNP** and **Ir₂@GSNP**, respectively) were prepared using a reverse microemulsion (water-in-oil) method, where water droplets are dispersed as nanosized liquid entities in a continuous domain of oil, and stabilized by surfactant molecules at the water/oil interface [21]. In order to synthesize the gold nanoparticles, quaternary water/oil (W/O) microemulsion was prepared by mixing 7.2 mL of Triton X-100 (surfactant), 7.2 mL of n-hexanol (co-surfactant), 30 mL of cyclohexane (oil) and a water solution consisting of a mixture of 1.8 mL $HAuCl_4·3H_2O$ (12.75 mM) water solution, 1.8 mL MES (36.5 mM) water solution and 0.6 mL $NaBH_4$ (423 mM) water solution. After 5 min, 0.1 mL of the corresponding metal complex solution – obtained by direct dissolution of 1 mg of each complex in 0.1 mL of water (for the Ir(III) complexes) or DMSO (for the Ru(II) complexes) – was added to the microemulsion, followed by the addition of 0.020 mL of APTES and 0.300 mL of TEOS. After 30 min, the silica polymerization reaction was completed by adding 0.160 mL of

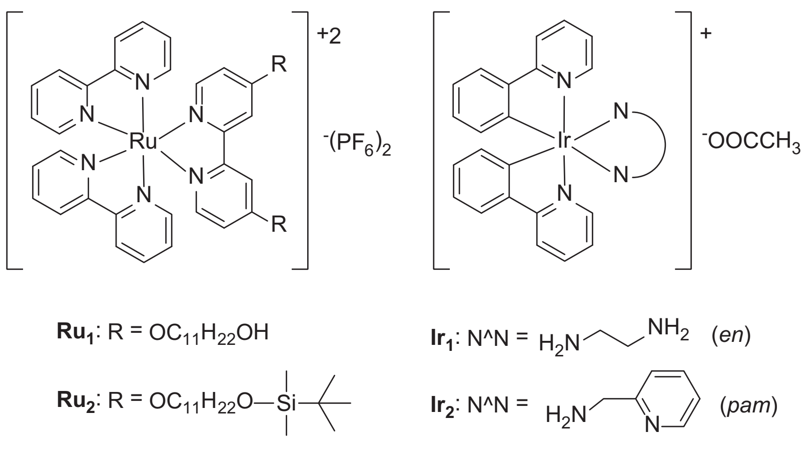

Chart 1. Chemical structures of the octahedral ionic Ru(II) and Ir(III) complexes.