



Hybrid systems based on gold nanostructures and porphyrins as promising photosensitizers for photodynamic therapy



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ABSTRACT

Gold nanostructures of two different shapes (spheres and rods) were synthesized to form a colloidal hybrid system with 5,10,15,20-tetrakis(*N*-methylpyridinium-4-yl)porphyrin tosylate salt ($\text{H}_2\text{TM4PyP}(\text{OTs})_4$) (POR) for applications in photodynamic therapy (PDT) using light in the visible spectral range. Electron paramagnetic resonance (EPR) experiments in combination with spin trapping were used for the detection of reactive oxygen species (ROS) and evaluation of the efficiency of these novel hybrid systems as photosensitizers. It is shown that the hybrid system consisting of gold nanorods (AuNR) and porphyrin (POR) is by far more efficient than its isolated components. This enhanced efficiency is explained by a synergetic effect between the AuNR and the porphyrin, wherein a rapid energy transfer from the former to the latter produces a large amount of singlet oxygen followed by its conversion into hydroxyl radicals. The mechanism was investigated using different spin traps and different ROS inhibitors. On the other hand, spherical gold nanoparticles (AuNP) do not show this synergetic effect. The synergetic effect for gold nanorods/POR hybrid is attributed to a larger field enhancement close to the gold nanorod surface in addition to the electrostatic attraction between the components of the hybrid system.

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1. Introduction

Photodynamic therapy (PDT) is a minimally invasive approved modality for clinical treatment of several types of cancer. The most accepted and wider use of PDT is for non-melanoma skin cancers [1]. It can, however, also be used in other non-malignant diseases in dermatology, ophthalmology, cardiology [2] and antimicrobial applications [3]. PDT consists mainly in topic or intravenous administration of a non-toxic drug or dye, the photosensitizers (PS). These compounds can selectively accumulate in tumor tissues and then be activated by light to generate reactive oxygen species (ROS) in the presence of molecular oxygen, mainly singlet oxygen, superoxide and hydroxyl radicals which are responsible for malignant cell death [4]. The efficacy of this process relies on the activation of the

PS by visible light preferentially in the biological optical window in the near infrared (700–1350 nm). In this region, light can penetrate more deeply into tissues because fat, water and hemoglobin have small absorptions [2,5]. An efficient PS requires chemical purity, physical and chemical stability, good biocompatibility, low toxicity in dark and rapid clearance from the body [1,6–9]. The most common photosensitizers used in clinical PDT are porphyrins, chlorins and phthalocyanines [2,5,10].

The mechanism involved in the light activation of the photosensitizers consists in light absorption and energy or charge transfer. The PS in the ground state possesses two electrons with opposite spin in a low energy molecular orbital, forming a singlet state. When light reaches the PS, photons are absorbed and one of these electrons is promoted to a higher energy electronic state keeping the spin orientation, the excited singlet state. This state is a short-lived state and it can return to the ground state losing its energy in processes of fluorescent emission, internal conversion of heat or in a process called intersystem crossing characterized by the inversion

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of the electronic spin (excited triplet state). In the triplet state, with two parallel spins, the PS may participate in two mechanisms for the generation of oxidative species: in the type I reaction the PS interacts with the substrate, such as cell membrane, by electron abstraction leading to superoxide species ($O_2^{\cdot-}$), hydroxyl (OH^\bullet), and/or peroxy radicals (OOH^\bullet). In type II mechanism the PS transfers its energy to molecular oxygen generating the excited state of molecular oxygen, called singlet oxygen (1O_2) which is highly reactive [1,4,5,11].

Metal nanoparticles can be useful for both fluorescence imaging and to enhance the photodynamic activity for singlet oxygen generation, increasing the triplet yield of the PS through coupling to surface plasmons [12]. The collective oscillation of surface electrons of metallic nanoparticles under light excitation result in enhancement of a wide range of optical process near the surface of the metallic nanoparticle such as surface-enhanced Raman scattering (SERS), fluorescent and phosphorescent emission, absorption, and metal enhanced singlet oxygen generation [13,14]. Although metal enhancement effect of metallic nanoparticles is well known for applications in metal-enhanced fluorescence (MEF) [15,16] it is not limited to fluorescence and the enhancement of singlet oxygen generation when in close proximity to a metal is already reported [12,13,17–19]. For example, an enhancement effect was presented using silver island or other metal films but for biological applications these films are not suitable. In this scenario, the synthesis and applications of metal nanostructures are more appropriate. Mooi and Heyne synthesized core-shell (Ag-SiO₂) nanoparticles with a covalently attached photosensitizer (Rose Bengal) to study the generation of singlet oxygen [19]. Planas et al. showed a dependency of singlet oxygen generation in a system with Rose Bengal tethered to the surface of core-shell silver-silica nanoparticles [20]. The authors showed that the silver core enhances the singlet oxygen generation through enhanced absorption of light and because it also enhances the radiative decay of 1O_2 , i.e., there is a boost in its phosphorescence emission and in its detection limits in biological systems.

Gold nanostructures (nanorods, spherical gold nanoparticles) have been widely used because of good biocompatibility, low cytotoxicity and facile synthesis with tunable plasmon resonance bands [21–24]. For example, spherical gold nanoparticles have been used as vehicle to deliver the PS [24], in which 5-aminolevulinic acid was conjugated with positively charged gold nanoparticles by electrostatic interaction and delivered preferentially into fibrosarcoma cells leading to enhanced reactive oxygen species generation. The first report of PDT mediated only by gold nanorods was made by Vankayala and co-workers [25]. In that study the authors demonstrated that gold nanorods itself can generate singlet oxygen and mediate PDT for tumor cell damage in mice under very low doses of single photon excitation in the absence of additional organic photosensitizers.

Porphyryns are well-known photosensitizers in PDT applications with excellent properties, such as high molar absorption coefficients in the visible spectral region, high quantum yield of triplet state and long-lived excited state (1 μ s), low cytotoxicity in dark, which make these compounds good candidates for PDT [2,26]. In the present work the synthesized water-soluble 5,10,15,20-tetrakis(*N*-methylpyridinium-4-yl)porphyrin tosylate salt ($H_2TM4PyP(OTs)_4$) (POR) in hybrid systems with gold nanorods and gold nanospheres was evaluated concerning its photodynamic activity and compared with commercial Verteporfin.

The detection of singlet oxygen is generally done by monitoring its phosphorescent emission at 1270 nm [20,27,28]. In this work we used spin trapping coupled to electron paramagnetic resonance (EPR), a very sensitive technique to detect reactive oxygen species, which is capable to identify and quantify the amount of singlet oxygen generated as well other radical species [29].

2. Experimental section

2.1. Porphyrin synthesis

The 5,10,15,20-tetrakis(*N*-methylpyridinium-4-yl)porphyrin tosylate salt [$H_2TM4PyP(OTs)_4$](POR) was prepared via alkylation of the parent *meso*-tetrakis(4 pyridyl)porphyrin according to an adaptation of the method of Safar et al. [30]. Verteporfin (VER), a derivative benzoporphyrin, is a commercial FDA approved benzoporphyrin derivative used in PDT and was used for comparison. It was obtained from Sigma-Aldrich, and used without purification.

2.2. Materials

The spin traps TEMP (2,2,6,6-tetramethylpiperidine) and PBN (*N*-*tert*-Butyl- α -phenylnitron), the free radical TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl), *D*-mannitol (C₆H₁₄O₆), sodium azide (NaN₃), dimethyl sulfoxide (DMSO), HAuCl₄, sodium citrate, NaBH₄, hexadecyltrimethylammonium bromide (CTAB), AgNO₃, ascorbic acid (C₆H₈O₆) were purchased from Sigma Aldrich, and used without purification.

2.3. Synthesis of citrate stabilized gold nanoparticles (AuNP-cit)

The method to synthesize gold nanoparticles with sodium citrate used in this work was described elsewhere [31]. Briefly, 250 μ L of HAuCl₄ (0.1 M) was added to 50 mL of deionized water at approximately 80 °C under vigorous stirring and the solution was heated to the boiling point. Then, 5 mL of aqueous solution of sodium citrate (1% in mass) was dropped into the gold compound solution. After a few minutes the solution changed from dark purple to a red wine color indicating the AuNP formation.

2.4. Synthesis of positively charged gold nanoparticles (AuNP-cys)

To synthesize positively charged spherical gold nanoparticles we used a protocol described by Jv et al. [32] as an adaption of the method proposed by Niidome and co-workers [33] based on NaBH₄ reduction of HAuCl₄ in the presence of cysteamine in contrast to those synthesized by citrate reduction of HAuCl₄ that presents a negative surface charge. Briefly, 570 μ L of HAuCl₄ (0.1 M) was added to 40 mL of deionized water at room temperature and then 400 μ L of an aqueous solution of cysteamine (213 mM) was dropped into this solution under stirring. After 20 min of stirring, 10 μ L of a freshly prepared NaBH₄ (10 mM) aqueous solution was added under vigorous stirring for 10 min. The average diameter of the nanoparticles is about 34 nm [32,33].

2.5. Synthesis of gold nanorods

Seed-mediated growth in the presence of the cationic surfactant hexadecyltrimethylammonium bromide (CTAB) has been continuously improved since its first demonstration by Jana et al. [34,35] and Nikoobakht and El-Sayed [36]. The metal salts are reduced in water at room temperature, with a strong reducing agent such as sodium borohydride (NaBH₄) to yield 3 nm spherical “seed” particles. For this, 10 mL of 0.1 mM of HAuCl₄ was mixed with 0.2 M CTAB solution. A 0.5 mL portion of fresh 0.01 M NaBH₄ was injected into the Au(III)-CTAB solution under stirring. The solution color changed from yellow to brownish-yellow. The seed solution was aged at room temperature for 1 h before use. In order to prepare the growth solution, 1.8 g of CTAB and 1 mL of sodium oleate (0.1 M) were dissolved in 50 mL of warm water (60 °C), according to the report by Ye et al. [37,38]. The solution was allowed to cool to 30 °C, when 2.4 mL of 4 mM AgNO₃ aqueous solution was added. The mixture was kept undisturbed at 30 °C for 15 min, after which 500 μ L of

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