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Antibacterial efficacy of acridine derivatives conjugated with gold nanoparticles

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ABSTRACT

Adsorption of acridine derivatives viz. 9-aminoacridine hydrochloride hydrate (9AA-HCl), acridine yellow (AY), acridine orange (AO), and proflavine (Pro) on citrate stabilized gold nanoparticle surface were studied using different analytical techniques like UV–vis absorption spectroscopy, Fourier transform infrared spectroscopy (FT-IR), and transmission electron microscopy (TEM). The amine moiety of acridine derivative binds strongly to the gold nanoparticles as confirmed by spectroscopic studies. The plasmon band observed for the wine red colloidal gold at 525 nm in the UV–vis spectrum is characteristic of gold nanoparticles. However, with the addition of acridine derivatives the intensity of the absorption band at 525 nm decreases and a new peak emerges at red-end region – a signature of formation of gold-drug complex. The TEM images show the average size of citrate stabilized gold nanoparticles as 15–20 nm, which becomes larger in the presence of various drugs due to aggregation. From the thermogravimetric analyses (TGA) we have measured the number of drug molecules attached per gold nanoparticle (AuNP). These gold nanoparticles are very important as drug delivery vehicles and for clinical applications it is necessary to understand their activity in vivo. The antibacterial efficacy of drugs coated gold nanoparticles were studied against various strains of Gram positive and Gram negative bacteria. Among the four drugs, 9AA-HCl and AO showed antibacterial activity and for both of them the AuNP conjugated drug showed better antibacterial efficacy than the bare drug. Because of the high penetrating power and large surface area of Au(0), a single gold nanoparticle can adsorb multiple drug molecules, hence this total entity acts as a single group against the bacteria.

1. Introduction

In recent years, acridine and its derivatives have attracted much attention owing to their immense pharmacological importance (Kragh-Hansen, 1981; Li et al., 2006; Salman et al., 2000). 9-Aminoacridine hydrochloride hydrate (9AA-HCl), acridine yellow (AY), acridine orange (AO), and proflavine (Pro) are the important fluorescent dyes belonging to those families. These dyes and their derivatives are extensively used in biological fields owing to their strong interaction with biomolecules such as DNA and proteins (Bentin and Nielsen, 2003; Kononov, 2001; Lee and Galley, 1988; Long et al., 2007; Lyles and Cameron, 2002; Veldhuyzen et al., 2003; Wesley et al., 1990) and their photochemical reactions in different media (Junior et al., 2003). Additionally, these acridine-based drugs are well-known for their antifungal, antibacterial, and antimalarial properties (Loechler and King, 1986; Zhu et al., 1994). These dyes also act as model photosensitizers in photodynamic therapy (PDT) and thus have drawn the interest of researchers (Schmidt et al., 1981). Among them, 9AA-HCl has been proposed as a specific fluorescent probe capable of binding the active center of guanidine benzoatases (GB) (Murza et al., 2000). In fact, it has been used to locate malignant cells in many tumor tissues (Steven et al., 1993) and used as fluorescent markers for detecting and inhibiting tumors. On the other hand AO is useful for studying the photophysics and molecular dynamics of DNA (Brauns et al., 1998). AO is also used as biological stains in fluorescence microscopy (Petit et al., 1993). The spectroscopic and photophysical properties of these acridine derivatives in homogeneous and micro heterogeneous media are very helpful for a better understanding of the nature of binding and biodistribution of this kind of dye inside the living cells. In our

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earlier work we have already studied the photophysics of 9AA-HCl in different micellar environments (Mitra et al., 2013).

Now a days, nanotechnology offers tremendous potential for medical diagnosis and therapy as it bears a successful impact on biological and medicinal processes occurring at nanometer length (West and Halas, 2000; Zandonella, 2003). Recently, gold nanoparticles (AuNPs) based drug/gene delivery systems have attracted attention due to their functional versatility, biocompatibility, and low toxicity (Bhattacharya and Mukherjee, 2008; Connor et al., 2005; De et al., 2008; Templeton et al., 2000; Vigderman and Zubarev, 2013). AuNPs are multipurpose agents with numerous applications in biomedicine like diagnostic assay (Goodman et al., 2004), thermal ablation, radiotherapy enhancement (Hainfeld et al., 2004; Hirsch et al., 2003) as well as used for the detection of antigen in conjugation with an antibody (Nam et al., 2003). The size of the AuNPs can be tuned from 2 to 100 nm with correspondingly large surface-to-volume ratios (Daniel and Astruc, 2004). In addition, the shape and the orientation can also be manipulated by applying different techniques such as polarization dependent two-photon luminescence (TPL), etc. (Senyuk et al., 2012). Due to their large surface area to volume ratio and biocompatibility, AuNPs are considered as ideal candidates for carrying large amounts of antibiotics without compromising their activity and also significant progress was observed in the delivery of large biomacromolecules, including DNA, siRNA, and proteins (Aryal et al., 2009; Ma et al., 2009; Pornpattananangkul et al., 2010; Wijaya et al., 2009; Yavuz et al., 2009). Many reports are available in the literature on the antimicrobial effect of antibiotics conjugated with different nanoparticles (Grace and Pandian, 2007; Gu et al., 2003; Li et al., 2005; Nepal et al., 2008; Rosemary et al., 2006; Saha et al., 2007). The advantage of using both antibiotics and inorganic nanoparticles together is that if bacteria have resistance against one of the components, a further component could kill them in a different manner. As an example, the antimicrobial activity of vancomycin coated gold nanoparticles gets enhanced (Gu et al., 2003). Similarly the coatings of aminoglycosidic antibiotics on gold nanoparticles have an antibacterial effect on a range of Gram-positive and Gram-negative bacteria (Grace and Pandian, 2007; Saha et al., 2007). On the other hand, GNPs have been used in photothermal therapy for the destruction of cancer cells or tumors. When irradiated with a focused laser in the near-infrared region (NIR) of suitable wavelength, targeted aggregates of GNPs, nanorods, or nanoshells can kill bacteria (Zharov et al., 2006) and cancer cells (Zharov et al., 2005).

In our present study, the conjugation of 9AA-HCl, AY, AO and Pro with citrate stabilized gold nanoparticles have been studied by various analytical techniques like UV–vis spectrum, Fourier transform infrared spectroscopy (FT-IR) and transmission electron microscope (TEM). Further, the antibacterial efficacy of drugs coated gold nanoparticles were studied against strains of Gram positive and Gram negative bacteria. Thus, the study of conjugation of these biologically important drugs with gold nanoparticles (AuNPs) as exogenous and endogenous drug-delivery vehicles is of utmost importance.

2. Materials and methods

2.1. Materials

$HAuCl_4 \cdot 3H_2O$, trisodium citrate were purchased from Sigma. 9AA-HCl, AY, AO and Pro were also purchased from Sigma–Aldrich. Potassium bromide (spectroscopic grade), used for infrared studies, was purchased from Merck. All the solvents were purchased from commercial sources and distilled prior to use. Double distilled deionized water was used for all the experiments.

2.2. Preparation of citrate capped gold nanoparticles (Turkevich et al., 1951)

To a boiling solution of $HAuCl_4 \cdot 3H_2O$ (1 mM, 0.5 cm^3), trisodium citrate (10^{-2} M, 0.5 cm^3) and double distilled water (18.5 ml) was added as one portion and after the addition, the previously yellow solution of gold chloride turned wine red in color.

2.3. Preparation of antibiotic coated gold nanoparticles

The drugs coated gold nanoparticles were prepared as follows: 0.25 mM citrate stabilized gold nanoparticles was mixed with drugs in water and stirred effectively for 2 h. This is marked as the standard sample. Similarly, antibiotic protected Au(0) were prepared at two different concentrations of gold particles viz. 0.5 mM and 1.0 mM to study the function of nanoparticles on the microbial activities.

2.4. Details of microbial assay

Antibacterial activity was studied by determining the zone of inhibition following standard agar diffusion method. From overnight cultures of *E. coli* (Gram −ve) and *B. subtilis* (Gram +ve) strains, next day secondary cultures were inoculated at a dilution of 1:100 in LB and allowed to grow at 37 °C in shaking condition to reach the OD_{600} to 0.6. Bacterial suspensions from these secondary cultures were then used for 90 mm diameter petri dish filled with 25 ml LB-agar. After punching wells in the agar plates, 20 μl of each drug and gold nanoparticle conjugated drug were used to fill these wells and the zone of inhibition was measured in the next day after incubating the plates at 37 °C for overnight.

For TEM image, 400 μl of the above *B. subtilis* cell suspension was centrifuged and the cell pellet was washed twice with 1X phosphate buffer saline (PBS). The cells were then fixed with 4% para-formaldehyde for 10 min at 37 °C followed by washing with 1X PBS thrice. After re-suspension, the cells were allowed to attach on the grid, air dried and proceed for image acquisition.

2.5. Apparatus

The absorption spectra were recorded on a Jasco V-650 absorption spectrophotometer at 298 K within a wavelength range of 300–800 nm using a pair of 1 cm × 1 cm path length quartz cuvettes. For TEM imaging, the samples were observed in transmission electron microscope (FEI model Technie G2 20S). The infrared spectra were recorded using vacuum dried samples in the form of KBr pellets in PerkinElmer Spectrum 100 FT-IR Spectrometer. Thermogravimetry (TG) measurements were carried out using a thermogravimetry analyzer (Netzsch, Germany, model: STA 449C) at a heating rate of 10 °C/min. Thermogravimetry analysis (TGA) is a highly useful analytical technique which was performed with all the samples to understand the nature and strength of binding of drugs with the gold nanoparticles (AuNPs). Through combining TGA with size analyses of the nanoparticles by TEM, the number of ligands attached to AuNPs can be determined.

3. Results and discussion

The colloidal solution containing citrate capped gold nanoparticles has very intense and characteristic wine red color originating from the coherent electron motion. The plasmon band observed for the wine red colloidal gold at 525 nm in the UV–vis spectrum is characteristic of gold nanoparticles as depicted in Fig. 1. With the addition of acridine derivatives (9AA-HCl, AY, AO, and Pro) the intensity of the absorption band at 525 nm is decreased and accompanied by a new peak emerging at red-end region due to the

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