Colloids and Surfaces A: Physicochem. Eng. Aspects 511 (2016) 264–271

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

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

Gold nanohybrid systems with tunable fluorescent feature: Interaction of cysteine and cysteine-containing peptides with gold in two- and three-dimensional systems

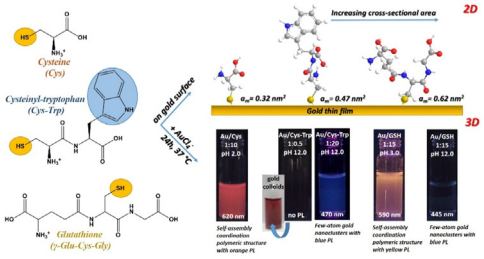
Edit Csapó[a,b,*], Ditta Ungor[a], Ádám Juhász[a], Gábor K. Tóth[b], Imre Dékány[a,b,*]

[a] MTA-SZTE Supramolecular and Nanostructured Materials Research Group, University of Szeged, H-6720, Dóm tér 8, Szeged, Hungary
[b] Department of Medical Chemistry, Faculty of Medicine, University of Szeged, H-6720, Dóm tér 8, Szeged, Hungary

HIGHLIGHTS

- Adsorbed amount of Cys, Cys-Trp and GSH on gold film was measured by SPR and QCM.
- Measured cross sectional area of ligands are in good agreement with calculated data.
- Self-assembly fluorescent CPs structure of Au/Cys and Au/GSH was proven at acidic pH.
- Presence of Trp prevents the formation of yellow-or orange-emitting CPs structure.
- Interaction of $AuCl_4^-$ with Cys-Trp results in blue-emitting Au NCs and colloid Au NPs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 September 2016
Received in revised form
30 September 2016
Accepted 5 October 2016
Available online 5 October 2016

Keywords:
SPR
QCM
Gold nanoparticles
Gold nanoclusters
Tunable photoluminescence
Self-assembly structure

ABSTRACT

Interactions of $_L$-cysteine (Cys), $_L$-cysteinyl-tryptophan (Cys-Trp) and $_L$-glutathione (γ-Glu-Cys-Gly, GSH) with gold-coated surface (Au^0) and tetrachloroaurate ions ($AuCl_4^-$) were studied in two(2D)- and three(3D)-dimensional systems, respectively. The irreversible bindings of the molecules onto gold surface via gold-sulphur (Au-S) bonds were proven by 2D quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) techniques. Moreover, the cross-sectional areas and the possible orientation of these biomolecules on gold film were also determined and compared with the theoretical calculated values. The spontaneous interactions of Cys, Cys-Trp and GSH with $AuCl_4^-$ were studied in aqueous medium at 37 °C as well. Depending on the pH and the molar ratios of $AuCl_4^-$/molecules, the formation of diverse gold nanostructures were confirmed. It was proven that the spontaneous interactions of $AuCl_4^-$ with Cys and GSH result in the formation of orange- and yellow-emitting gold nanohybrid systems ($\lambda_{emission}$ = 620 and 590 nm) consist of a self-assembly structure at acidic conditions. In contrast, at alkaline medium fluorescent Au nanostructures were not formed in the $AuCl_4^-$/Cys system, but ultrasmall blue-emitting gold nanoclusters (Au NCs) were identified in the samples containing $AuCl_4^-$/GSH. For $AuCl_4^-$/Cys-Trp systems the presence of Trp aromatic side chain prevents the formation of

* Corresponding authors at: MTA-SZTE Supramolecular and Nanostructured Materials Research Group, University of Szeged, H-6720, Dóm tér 8, Szeged, Hungary.
E-mail addresses: tidecs2000@yahoo.co.uk (E. Csapó), i.dekany@sol.chem.u-szeged.hu, i.dekany@chem.u-szeged.hu (I. Dékány).

http://dx.doi.org/10.1016/j.colsurfa.2016.10.003
0927-7757/

the fluorescence self-assembly nanostructure and depending on the pH, Au concentration and ratio of the reactants the appearance of dipeptide-stabilized plasmonic gold nanoparticles (Au NPs) or supramolecular gold complexes having intense blue fluorescence (λ = 470 nm) were observed.

1. Introduction

The biocompatible preparation routes of plasmonic Au NPs and ultra-small Au NCs having highly fluorescent features are focus of extensive researches. Protein-based (*e.g.* bovine serum albumin, lysozyme, pepsin, tripsin *etc.*) Au NPs and NCs have been synthesized and characterized previously [1–5] but the number of articles relating to the small peptide- or amino acid-reduced Au nanostructures are somewhat less [6–8]. The unique fluorescence of sub-nanometer sized Au NCs (d = 0.3–2 nm) depends on various factors like number of Au atoms in the cluster, valence state of Au, surface-bonded ligands, crystallinity of the particles *etc.* [3]. The Au NCs containing Au^0 in the core have been used to develop optical probes for biosensing, biolabelling and bioimaging applications [3,5,9,10]. Besides Au^0-based fluorescent nanostructures, the metal ion-containing inorganic-organic hybrid multilayer structures or co-called "coordination polymers" are also possess intense fluorescence. These nanosized hybrid materials featuring the periodic and infinite build-up metal ions and ligand via coordination interactions [11]. Cu(I)-, Pb(II)-, Zn(II)- or other metal ion-based coordination polymers were synthesized previously [12–14], but Au(I) is also potent candidate for fabrication of fluorescent nanohybrid systems using biocompatible molecules. The Cys and the GSH biomolecules are widely used to functionalize the surface of Au NPs via the strong Au-S covalent bond [15–18]. Moreover, these molecules are able to reduce and coordinate with gold(I) ions to form complexes that are common intermediates in the preparation of thiol-protected Au NCs [5,19]. However, Söptei et al. proved that the interaction of $AuCl_4^-$ and Cys results the formation of Au(I)-Cys multilayer structures having orange photoluminescence (PL) but the dominant role of $AuCl_4^-$/Cys ratio as well as the effect of pH on the formation of self-assembly nanostructure were not investigated [20]. It is also well-known that in the $AuCl_4^-$/GSH system different Au NCs (Au_{10}-Au_{39}) can be fabricated in aqueous medium at pH 12 [21] but the influence of pH as well as the ratio of reactants were not investigated previously.

Numerous articles were published on the synthesis, characterization and biofunctionalization of gold nanoparticles. In the above mentioned articles the authors use both reducing (*e.g.* citrate, borohydride, hydrazine *etc.*) and stabilizing agents (*e.g.* biocompatible polymers) as well as functionalizing molecules (*e.g.* peptide, proteins *etc.*) in order to stabilize the prepared gold nanoparticles. In this article we use a simple amino acid and a di- and tripeptides to synthesize directly gold nanohybrid structures having tuneable optical features without application of any other reducing and stabilizing agents. This "one-pot" biocompatible synthesis route (spontaneous interaction of $AuCl_4^-$ ions with the studied molecules) results in the formation of diverse gold nanohybrid structures. The previously published results of Au/Cys and Au/GSH nanohybrid structures were completed with several structural as well as optical studies in order to optimize the preparation routes. Besides the Cys and GSH, a new dipeptide (Cys-Trp) was also synthesize in order to investigate the effect of the presence of Trp on the possible formation of self-assembly coordination polymer structure. The interaction of these three molecules with $AuCl_4^-$ were investigated at 37 °C in aqueous medium and the dominant role of pH as well as the influence of $AuCl_4^-$/ligand ratios on the formation of fluorescent gold nanostructures were studied. The binding capability of these molecules onto gold-coated surface was also studied to provide information on the cross-sectional area as well as the orientation of the bonded biomolecules on gold surface.

2. Materials and methods

2.1. Materials

All the chemicals were of analytical grade and were used without further purification. $_L$-Cys (99.5%) were purchased from Fluka, the $_L$-GSH reduced (≥98.0%) from Sigma-Aldrich, gold(III) chloride acid trihydrate ($HAuCl_4 \times 3H_2O$) from VWR International. The hydrogen chloride solution (HCl, 37%) and sodium hydroxide (NaOH, pastilles) were purchased from Molar. In all cases the stock solutions were freshly prepared using Milli-Q ultrapure water (18.2 MΩ cm at 25 °C).

2.2. Synthesis of Cys-Trp

The dipeptide (Cys-Trp) was synthesized in our laboratory by liquid-phase technique utilizing Boc chemistry. Briefly, 0.765 g (1.65 mmol) Boc-Cys(Trt)-OH was dissolved in 20 ml abs. ethylacetate and cooled to −15 °C. Under stirring 0.26 ml of isobutylchloroformate and 0.23 ml of triethylamine were added. After 25 min of stirring at −15 °C, 0.445 g of H-TrpOBu^t × HCl and 0.209 ml of triethylamine were added. The reaction mixture was stirred at 0 °C for 1 h. Stirring was continued at 25 °C overnight. The precipitated trimethylamine × HCl was filtered, the organic solution was extracted with 5% $KHCO_3$, 5% $KHSO_4$ and water. The solution was dried over sicc. Na_2SO_4 and the solvent was removed. The resulted crude protected dipeptide was treated with TFA/DTT/water (90:5:5), on RT for 2 h. Than the TFA was evaporated, the residue was triturated with ether and the resulted crude peptide was solubilized in 10% aqueous acetic acid, filtered and lyophilized. The crude peptide was purified by semipreparative RP-HPLC on a Phenomenex Jupiter Proteo (Torrance, California) C18 10 μm column (15 × 250 mm). The applied flow rate was 5 ml/min, the gradient was 10–40% in 60 min. The homogeneity of the resulted peptide was evaluated by analytical RP-HPLC on a 4.6 × 250 mm Phenomenex Luna 10 C18 100 column at 1.2 ml/min flow rate and a gradient of 18–33% in 15 min. R_t = 10.51. The peptide was further characterized by mass spectrometry using a Finnigan TSQ 7000 tandem quadrupol mass spectrometer equipped with electrospray ion source. The result is the following: $M_{w,calc}$ = 307.08, $M_{w,measured}$ = 308.1.

2.3. Syntheses of Au/Cys, au/Cys-Trp and Au/GSH systems

For syntheses of gold/biomolecule hybrid nanostructures the appropriate amount of $HAuCl_4$ solution was added in one step to the adequate amount of the aqueous solution of the studied molecules. The $AuCl_4^-$:biomolecules molar ratios were varied from *ca.* 1:1 to 1:40, the concentration of $AuCl_4^-$ was changed from 0.1 mM to 1.0 mM depending on the system. The syntheses were carried out at different pH (pH 1–12) and for structural characterization of the products an optimal pH value was used (Au/Cys pH 3.0;