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Studies on the Interactions of porous nano gold materials with protein using UV-VIS spectroscopy

Munmun Bardhan[1] and Tapas Misra[2]

[1]Department of Physics, Jadavpur University, Kolkata 700032, India
[2]Department of Physics, Sabang Sajanikanta Mahavidyalaya, Lutunia, Paschim Medinipur, India

Abstract

In this paper, the investigations on spectroscopic behavior of the binary complex of Porous nano Gold (PNG) with the protein human hemoglobin (Hb) by using UV-Vis steady state spectroscopy are reported. Study about the nature of complex (Hb•PNG) are the main aim of this observation. About 250 nm gold nano particles are synthesized by seed mediated method and characterised by TEM study. 1:1 ground state complex formation of Hb•PNG are confirmed by steady state absorption studies. There are many significant importance and applications in many drug formations and in drug delivery systems in human body.

Keywords: Binary complex, nano-particles , spectroscopy

1 Introduction

For nanoparticle bio-reactivity the main features are the interaction between nanoparticles and protein. The interaction plays an important role in biotechnology and biomedical application. The biological application of nanoparticles is expanding in food technology, cosmetics, medicine, pharmacology covering a wide range of area and also in drug delivery [1]. NP interacts with biomolecule such as protein, nucleic acid, lipids, and biological membrane due to their size and surface to mass ratio. The main key of the interaction is the adsorption of protein on the nanoparticle surface forming corona. This nano protein corona may influence the biological activity of the NP [2,3]. Porous nanomaterials are material made of framework supporting a regular porous structure. Porous materials have been emerged as one of the most promising candidates because of their biocompatibility, high surface to volume ratio, monodispersed, low-cost synthetic procedure and capability of efficient drug delivery due to easy loading and releasing of drug via its pore. The formation of interconnected porosity increases the surface to volume ratio which tunes the new surface and optical properties. These features may alter the biological interaction as the surface contacts with biological environment. Porous gold is one of important nanomaterials for biological interaction. It is more biocompatible than other noble metal and nontoxic, so causes little damage to living cells. It also attached to biomolecules very effectively. Human hemoglobin (Hb) is an important heme protein as it can bind oxygen. Hb has a quaternary structure characteristic of four subunit globular protein. Each subunit is made of protein chain with a non protein heme group. The four subunits are made of two alpha and two beta polypeptide chain. The alpha chain contains 141 amino acid residue and each beta chain contain 146 amino acid residues. In the present study, the synthesis, characterization of porous nanogold particle and the interaction between globular protein (human hemoglobin, Hb) and porous nanogold (PNG) has been reported. The detailed interaction study has been monitored through steady state UV-Vis absorption study on proteins as a function of nanomaterials concentration.

2 Materials and methods

Cetyltrimethylammonium chloride (CTAC, CH_3-$(CH_2)_{14}$-CH_2-$N(CH_3)_3^+Cl^-$), ethanol (EtOH, C_2H_5OH), Gold(III) chloride trihydrate ($HAuCl_4$: $3H2O$) was Sodium borohydride ($NaBH_4$, 99%), L-ascorbic acid (AA, 99.5+%), all are purchased from Sigma-Alrdich and used without further purification. Hb are also used from Sigma-Aldrich and tested be-

Email: bardhanm@gmail.com, tapas@sabangcollege.ac.in
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fore use for the impurity check. Millipore water (18.2 MΩ resistivity) was used to prepare solution for all experiments.

2.1 Synthesis of porous gold nanoparticle

Porous nano gold (PNG) was synthesized by seed mediated method [4].
Synthesis of Au seed: 10ml 0.10M CTAC solution was mixed with 0.25ml, 10mM HAuCl4 solution under magnetic stirrer. 0.30ml 10mM freshly prepared NaBH4 solution was quickly injected into the mixture. The solution was stirred for 1min and then left for 2h for ageing. The solution was diluted to 1000-fold with 0.10M CTAC solution and then it was used for the next step.
Synthesis of PNG: First a growth solution was prepared by adding 0.50ml, 10 mM HAuCl4 and 0.10ml, 0.10M AA into a 10ml 0.10MCTAC solution. The solution was stirred gently for 30s. Then 0.015 ml diluted Au seed solution was added to the mixture and gently mixed for another 30s. The solution left undisturbed at room temperature for 4hr. The solution was washed with water through centrifugation and collected PNG was dispersed in 5 ml water.

2.2 Transmission Electron Microscopy

TEM studies were carried out to measure the particle size of PNG. sample were mixed in appropriate quantity of EtOH and sonicated for 15 min to achieve a good dispersion of particles. A FEI, Tecnai G2 F30, S-Twin microscope operating at 300 kV for electron imaging is used. For the TEM measurement, 300 mesh copper formvar/carbon grids were used. The supernatants were then drop casted on carbon coated copper grid and the grids were dried for 24 hrs before taking the images.

2.3 UV Visible Spectroscopy

The UV-vis absorption spectra were recorded at room temperature on a JASCO V-650 spectrophotometer using a quartz cuvette of 1 cm optical path length.

3 Results and Discussion

3.1 Characterisation of nanoparticles

The prepared nanoparticles are characterized by TEM. PNGs are popcorn like and sizes are around 250 nm as shown from Fig 1.

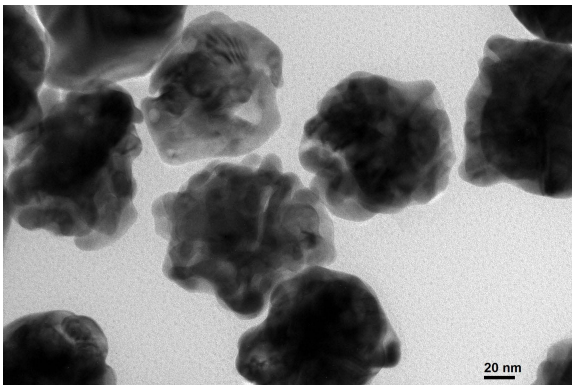

Figure 1: TEM images of PNG

Fig. 2 represents the absorption spectra of Hb in presence of PNG. Hemoglobin exhibits intense absorption in the region of wavelengths above 320 nm [5] Strong absorptions occur near 400 nm and this peak region is known as the Soret band [6,7] The Soret band is characteristic of hematoporphyrin proteins due to p-p* transition. [8,9]. This Soret band due to the heme group which is embedded in the hydrophobic pocket of proteins backbone through appropriate folding [10] The absorption spectra gradually decrease in presence with the increasing concentration of Porous gold nanoparticles (PNG) at 405 nm region while at longer wavelength region 600 nm, it gradually increases. Here a clear isosbestic point is also observed. The hypochromic effect (without ant spectral shift) for PNG (decrement of absorption spectra of Hb with the addition of PNG) indicates the possibility of formation of ground state complex. So the longer wavelength region absorption band (region 600 nm) is due to the ground state complex formation of Hb•PNG.

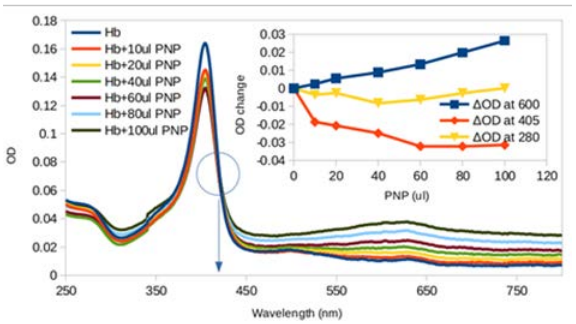

Figure 2: UV-Vis Absorption spectra of Hb with different concentrations of PNG

The inset (fig. 2) pic shows the change in optical density at three different wavelengths. To observe the stoichiometric ratio of the complex, the Benesi

and Hildebrand equation [11] can be utilized:

$$\frac{1}{(A_0 - A_{obs})} = \frac{1}{(A_0 - A_c)} + \frac{1}{k_A (A_0 - A_c)[PNG]} \text{----------(1)}$$

Where Aobs is observed absorbance of Hb solution containing different concentrations of PNG at 405 nm; A0 and Ac are the absorbance of free Hb and the complex solution at the same concentration of free Hb. A plot of reciprocal of change in optical density with reciprocal of concentration of PNG is obtained in Fig. 3.

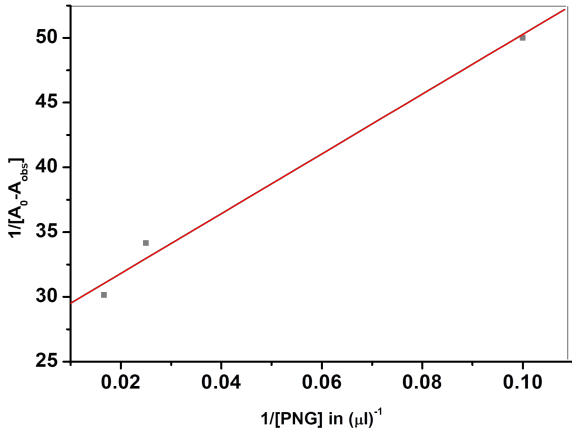

Figure 3: Benesi Hildebrand plot

A good linear dependency of this plot indicates 1:1 stoichiometry complex of Hb•PNG. This similar stoichiometry complex is observed for similar complex of Human Hemoglobin-Gold Nano Particle [12]. This 1:1 complex is very useful for various applications such as binding of drug, chemotherapy etc.

4 Conclusion

CTAC templated Porous nano gold particles are synthesized with an average size of 250 nm. Interaction study of human hemoglobin with this porous gold nanoparticles has been observed by UV-Vis spectroscopic technique. The steady state absorptions (UV-Vis absorption study) investigations indicates that a 1:1 ground state complex of Hb•PNG is formed in this study. The binding force of formation of ground state complex is of hydrogen bonding in nature. This ground state complex of porous nano gold with human protein has a potential application in drugs and drug delivery systems in human.

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