Binding interaction of a fluoranthene–thiol on gold nanoparticles with \(\beta\)-cyclodextrin and DNA

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\textbf{ABSTRACT}

In this paper, we report the preparation of a fluoranthene–thiol derivative, the assembly of the compound onto gold nanoparticles, and their binding to \(\beta\)-cyclodextrin and DNA. The synthesised fluoranthene–thiol is characterised using infra-red (IR), nuclear magnetic resonance (NMR), and mass spectrometric techniques. The gold nanoparticles are characterised using transmission electron microscopy and energy dispersive X-ray spectroscopy microanalysis. The size of the nanoparticles is 5 ± 1 nm. The thiol–gold nanoparticles bind to \(\beta\)-cyclodextrin, and DNA and the fluorescence spectra show enhancement of fluorescence on the binding. The thiol–gold nanoparticles form a host–guest complex with \(\beta\)-cyclodextrin and the binding constant of the complex is 1330 mol\(^{-1}\) dm\(^3\). This reveals the openness of the thiol on the surface of the gold nanoparticles.

\textbf{1. Introduction}

Gold nanoparticles (GNPs) in the 1–100 nm size range and fluorescent thiol molecules self-assembled on GNPs have emerged as a separate branch of nanoscience and technology. Gold is a stable noble metal at the nanoscale and designers of any nanodevice requiring metallic components consider gold favourably. Gold offers a unique chemistry that allows it to be used as a platform on which organic molecules self-assemble, usually bound sulphur atoms [1–3]. Such self-assembled structures may be used as sensitive biomedical and chemical sensors. Overall, a better understanding and control of the essential properties of GNPs may lead us to find a lot of amazing applications in microscopy labels, gene transcription, drug delivery, therapeutics, and bio-sensors [4–6]. GNPs can enter cells and this has resulted in the concentration of research on the attachment of various small molecules and biological macromolecules to gold in the view of combining functionality and transport. GNPs can potentially stabilise and protect DNA in solution. This leads to advantages over multistep methods involving separate methods on delivery and...
stabilisation [7,8]. Attaching molecules on the surface of GNPs can lead to designing DNA binders tethered with imaging and hyperthermia ‘nanohandles’ as the nanoparticles are known to have these characteristics [9,10].

β-Cyclodextrin (β-CD) is a cyclic oligosaccharide consisting of seven (α-1,4)-linked α-D-glucopyranose units and contains a lipophilic central cavity and a hydrophilic outer surface. Due to the chair conformation of the glucopyranose units, the CDs are shaped like a truncated cone. The internal cavity is hydrophobic in nature which is a key feature of the cyclodextrins providing the ability to form complexes, which include a variety of guest molecules [11–14]. CD can act as a fit-and-find detector of the openness of molecules on the surface of nanoparticles to bind to macromolecular targets like DNA [15,16]. Based on the above points, we carried out the experiments discussed in this paper. Herein, we discuss the strength of binding of a fluoranthene-based ligand-attached GNPs to β-cyclodextrin and DNA.

2. Materials and methods

2.1. Chemicals

3-Aminofluoranthene, 3-mercapto-2-butanone, and chloroaouric acid of the grade AnalaR were obtained from Sigma Aldrich. β-Cyclodextrin was the product of HiMedia. The solvents were purchased from Merck.

2.2. Methods

2.2.1. Synthesis of 3-(fluoranthen-1-ylimino)butane-2-thiol

0.25 gm of 3-amino fluoranthene and 0.2 ml of 3-mercapto-2-butanone were dissolved in 25 ml of methanol. The solution was stirred for 30 minutes at 550 rpm using a magnetic stirrer. A pellet of NaOH dissolved in 5 ml of methanol solution was added to the above solution. The temperature was maintained at 60 °C and the reaction was continued for a time period of 8 hours. Dark brown coloured crystals were separated by filtration and dried. The product (yield 65%) was purified by repeated crystallisation from methanol.

2.2.2. Formation of the thiol-attached GNPs

0.4 mmol of 3-aminofluoranthene and 3-mercapto-2-butanone inclusion were dissolved in 25 ml of methanol and stirred using a magnetic stirrer. 0.04 mmol of HAuCl₄ (chloroaouric acid) was added to the above solution and stirred for 15 minutes. 0.2 mmol of an ice-cold solution of NaBH₄ was added drop-wise to the above mixture. The mixture was stirred for 18 hours. Freeze drying method was used to remove the solvent. Yellow coloured colloidal crystals were obtained at the end of the process.

2.3. Binding studies

Stock solutions of β-CD and DNA were made in triply distilled water and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.4), respectively. Test solutions were made by appropriate dilution of the stock solutions. The solutions were made just before the spectral measurements.
2.4. Instrumentation

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker-AMX spectrometer operating at 400 MHz, using CDCl₃ as the solvent. FT–IR spectra were recorded using a Bruker-a spectrometer. The samples were prepared by grinding the compounds with KBr and preparing pellets. UV-Visible absorption spectra were recorded using a JASCO V-630 spectrophotometer. A JASCO 8000 spectrofluorometer was used to record fluorescence spectra. The energy dispersive X-ray spectrum was recorded using a JEOL JSM 6360 instrument. A minimum accelerating voltage of 15 KeV was applied to detect the presence of the possible elements. Transmission electron microscopic (TEM) images were recorded using a JEOL JEM 2100 transmission electron microscope.

2.5. Molecular docking

The size of the fused six-membered ring of fluoranthene was theoretically optimised from the software Schrödinger Impref minimisation (OPLS 2005) (Version 10.2). Molecular docking studies were performed using the software Schrödinger, a theoretical structure program based on quantum and molecular mechanics. The docking engine approximates an exhaustive search method with similarities to select and dock in different molecular pose. Grids were constructed which overlaid the binding site. Ligands root node was placed in the binding site and a set of diverse and energetically favourable poses were retained. The final set of poses was coarse minimised. The percentages of top ranking poses were predicted within certain defined root mean square deviation values from the original crystallographic pose.

3. Results and discussion

3-(Fluoranthen-1-ylimino)butane-2-thiol (Compound 1) was prepared as a simple imino derivative of 3-aminofluoranthene by treating the latter with 3-mercapto-2-butanone. The proton NMR spectrum of 1 is shown in Figure 1. The ¹³C NMR and the mass spectra of 1 are given in the Supporting Informations, SI1 and SI2, respectively. The prominent peaks of the C¹³ NMR recorded in the solvent dimethyl sulphoxide (DMSO) (signal centred at 40 ppm) are as follows: 147.57 ppm –C = N carbon of the imino group; 108–140 ppm – aromatic carbons; 28.96 ppm – methyl carbon at position 2; 18.94 – methyl carbon 4. The mass spectrum shows peak corresponding to the molecular ion at m/z = 303.2959. The base peak appears at m/z = 215.2833, corresponding to a fragment containing fluoranthene ring and the nitrogen. The infra-red (IR) spectrum of 1 showed prominent bands at 2925.23 (m), 2780.88 (m), and 1645.28 cm⁻¹ (s) corresponding, respectively, to the aliphatic C–H stretching, thiol S–H stretching, and C–N stretching of the imine bond (see SI3). The results evidently suggest that the Compound 1 has a structure as shown in Figure 1.

Chloroaурic acid was reduced in the presence of 1, with the assumption that the former would be reduced to GNPs by NaBH₄ and the Compound 1 would form a Au–S semi-covalent bond through its SH group. Sulphur–GNP interaction is in the order of 45 kcal/mol which leads to the formation of a stable, semi-covalent bond; in comparison, the C–C bond strength is ~83 kcal/mol [17]. GNPs were formed and the colloidal gold
sample thus formed was tested using energy dispersive X-ray technique (EDX). The EDX spectrum is shown in Figure 2. It shows the presence of GNPs along with the attached Compound 1. The size and the morphology of the GNPs were analysed using transmission electron microscope. Spherical thiol-covered GNPs could be seen (Figure 3). The average particle size was 5 ± 1 nm.

The UV-Visible absorption spectra of 1 assembled on GNP in the presence and the absence of β-CD in solution are shown in Figure 4(a). There are three bands, namely 242, 300, and a broad band which stretches up to 450 nm. These bands correspond to the π–π* transition, and surface plasmon resonance (SPR), respectively. Addition of β-CD in increasing concentrations leads to a hyperchromic shift of all these bands, the two shorter wavelength bands enhancing better than does the SPR band. The hyperchromic shift is due to the formation of host–guest complex between β-CD and 1–GNP. The fluoranthene ring gets encapsulated in the β-CD cavity as the width of the fused six-membered ring of 1 (5.7 Å) with the cavity size of β-CD (7.8 Å) [12,14]. The size of the fused six-membered ring fluoranthene is theoretically optimised from the software Schrödinger. The electronic transition probability of the guest molecule (1 on GNP) gets enhanced being inside the confinement of the β-CD cavity, leading to the free movement of electrons into various energy levels and hence an enhanced light absorption. There is also a 3 nm (indeed small) blue shift observed on the addition of β-CD. The blue shift is attributed to the dislodging of the fluoranthene structure from a polar environment to the nonpolar cavity of the β-CD. Such a blue shift is a typical observation in host–guest complex formation by CDs [18]. These results suggest that the complex formed is a host–guest complex.
Figure 2. EDX spectrum of 1–GNPs.

Figure 3. TEM image of 1–GNPs.
Figure 4. (a) UV-Visible absorption spectra of 1 assembled on GNP in the presence and the absence of β-CD in solution. (b) Fluorescence spectral changes of 1–GNP, on the addition of β-CD. (c) Benesi–Hildebrand plot of the binding of 1–GNPs to β-CD.
The changes of the fluorescence spectrum of 1–GNP, on the addition of β-CD, (Figure 4(b)) are more pronounced than those of the absorption spectrum. It is well known that fluorescence spectroscopy is more sensitive to changes in the local environment of compounds in solution. Addition of β-CD in step-wise increasing concentrations enhances the fluorescence of 1–GNPs. This is due to the restriction offered to the relaxation processes of the fluorophore by the β-CD cavity [19]. Hence, clearly a host–guest complex is formed between 1 on GNP and β-CD is formed. The enhancement of fluorescence is linear and the plot of $1/\left[I - I_0\right]$ vs. $1/I' - I_0$, following the modified Benesi–Hildebrand equation [20] (Equation 1) yields a straight line, as shown in Figure 4(c).

$$\frac{1}{I - I_0} = \frac{1}{I' - I_0} + \frac{1}{(I - I_0)K[\beta-CD]}$$

where $I$ refers to the intensity of fluorescence of the 1–GNPs at various concentrations of the added β-CD, $I'$ is the intensity at the largest concentration of β-CD, $I_0$ is the intensity of fluorescence of the fluorophore in water, and $K$ refers to the binding constant. Hence, it is comprehensible that the host–guest complex of 1–GNPs involves the binding interaction between one β-CD molecule and one fluoranthene unit present in the guest. 1:1 stoichiometric binding between the surface bound molecules and the host molecules has been observed in the case of nanoparticles surface modified with aromatic molecules and is observed with linearity in the Benesi–Hildebrand plot [15,16,18]. Rationally, the end moiety of 1, which is the fused six-membered aromatic ring, is likely to bind to the cavity of β-CD. The point to be noted here is that the imino nitrogen and the sulphur of the thiol function are bare towards the solvent molecules as the β-CD molecule does not interact with them. The calculated binding constant of the β-CD–1–GNP complex is 1330 mol$^{-1}$ dm$^3$.

In case where the bulky aromatic fused ring system, i.e. the fluoranthene is densely packed and available for interaction with targets, the β-CD cannot encapsulate it due to steric factors involved in hindering the crowded β-CD-capped monolayer of fluoranthene on GNPs in forming a complex. But, since the β-CD is found complexing the fluoranthene, it gives an indirect evidence that the molecule can bind to macromolecular targets like DNA.

Small molecules bind to DNA and alter their thermodynamic stability and functional property. The normal modes of binding are groove-binding and intercalation [21]. These bindings can be studied using spectroscopy [22,23]. We studied the binding of 1–GNPs to calf thymus DNA. The absorption spectra of the titration of 1–GNP against DNA are shown in Figure 5(a). The concentration of the compound was fixed at $2.27 \times 10^{-6}$ mol dm$^{-3}$, and the 1–GNP was added in aliquots. As the added concentration of 1–GNP increased, the absorbance showed an enhancement. Figure 5(b) shows the fluorescence spectra of 1–GNP on the step-wise addition of DNA. The intensity of fluorescence got enhanced on DNA binding. An enhancement of fluorescence is a result of the slowdown of molecular motions due to the binding to the biomolecule and non–radiative energy loss [24]. This observation is considerable to infer that the ligands on the GNPs are available for binding to DNA, even though they are attached to the surface of GNPs.
In order to perform molecular docking, the atoms and the binding sites were defined initially. The three-dimensional structures of the DNA and the ligand were fed into the Schrödinger and the binding sites were studied. The ligand (Compound 1) was introduced and docking calculation was allowed to run using shape-based search algorithm and the A-score scoring functions. The scoring function is responsible for evaluating the energy between the ligand and the DNA molecule. Flexible docking was allowed by constructing grids over the binding sites and energy-based rotation was set for that ligand group of atoms that did not have rotatable bond. For each rotation, torsions were created and poses (conformations) were generated during docking. The best docking model was selected according to the lowest A-score calculated by the software. The most suitable binding conformation was selected on the basis of bonding interactions between the DNA and Compound 1 near the suitable binding site. The lowest energy poses indicated the

Figure 5. (a) UV-Visible spectra of 1–GNP vs. DNA binding titration. (b) Fluorescence spectra of 1–GNP in the presence of various added amounts of DNA.
highest binding affinity. Docking pose 1 (as shown in Figure 6) corresponds to the best binding affinity of Compound 1 with DNA, with a binding energy of $-4.2895$ kcal mol$^{-1}$ with respective atomic interaction overlay of S–H⋯N hydrogen bond length 2.120 Å, C–N⋯H hydrogen bond length of 2.319 Å. The value reveals that hydrogen bonding binding has a major contribution in binding of Compound 1 to DNA.

4. Conclusion

3-(Fluoranthen-1-ylimino)butane-2-thiol is synthesised and characterised using IR, NMR, and mass spectroscopic techniques. Its attachment to gold nanoparticles, done on in situ the reduction of HAuCl$_4$, is studied. The fluoranthene moiety is assembled as the monolayer on GNP. The GNP surface-attached molecule binds to β-cyclodextrin and DNA. β-Cyclodextrin forms a host–guest complex with the thiol on GNP and hence the thiol molecule is found capable of binding to targets. Studies on this kind of host–guest binding to β-cyclodextrin and DNA binding of the molecules on GNP can lead to the understanding of hyperthermia nanohandles carrying fluorescent reporter molecules and their openness to bind to targets.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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