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Estimation of biogenic amines and biothiols by metal complex of fluorescent organic nanoparticles acting as single receptor multi-analyte sensor in aqueous medium

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ABSTRACT

A novel chemosensor based on metal complex of fluorescent organic nanoparticles (FONs) of new receptor (**1**) with ferric ions has been developed through simple synthetic procedure. The prepared receptor and its complex with ferric ions were subjected to FONs using re-precipitation method. FONs (**N1**) and FONs-Fe^{3+} ensemble (**N1.Fe^{3+}**) were characterized using various techniques such as NMR, EDAX and DLS. The **N1.Fe^{3+}** were further investigated for their sensor properties and found them selective for determination of tyramine and 4,6-diamino-2-mercaptopyrimidine, having no interference from any other biogenic amines or biothiols. **N1.Fe^{3+}** showed a detection limit as low as 4.95 nM and 3.02 nM respectively for tyramine and 4,6-diamino-2-mercaptopyrimidine, fulfilling the concept of "Single receptor multianalyte determination".

1. Introduction

Research on major classes of biomolecules such as amino acids, biogenic amines, nucleic acids, nucleosides, carbohydrates, biothiols and proteins has gained a vast attention in the recent years due to potential applications offered by these biomolecules towards biology, medicine and food safety [1–4]. For example, amino acids act as important metabolic intermediate in human body; protein kinases are regarded as the major target for drug discovery and as biomarkers for cancer diagnosis; human serum albumin (HSA) plays vital physiological functions of transporting hormones and amino acids, carbohydrates are involved in many biological processes of metabolism and cell structure [5–10]. Thiols, which are present in trace amount in natural samples found their extensive use in cosmetic products, vulcanization processes, preparation of pesticides, drugs for the treatment of hypothyroidism, as biomarkers and as antioxidants in food materials, etc. [11–14]. Beside these significant roles, biothiols also depict crucial role in many biological processes such that alterations in their level can lead to various diseases like liver damage, oedema, Alzheimer's disease, cardiovascular disease, osteoporosis, cancer and AIDS [15–17]. Similarly, amines play equally vital part in physiological system by acting along with amino acids as neurotransmitters that enables the generation of chemical signals in nervous system and includes norepinephrine, histamine, dopamine, β-phenylethylamine, tyramine, tryptamine, etc. [18,19]. Biogenic amines like spermine, spermidine, putrescine and cadaverine regulates the function of nucleic acids, protein synthesis and membrane stabilization and are involved in antioxidant activity. Generally, their presence can be expected in all food items, which contain protein or amino acids having tendency of undergoing microbial or biochemical activity. However, higher level of content is unenviable in foods and beverages due to development of several allergic disorders [20–24]. Thus, focusing on the importance of thiols and amines related to food quality control and human health concern, design of analytical methods for the estimation of amino and mercapto biomolecules in biological and environmental samples have become an important area of research. Traditionally, high performance liquid chromatography (HPLC), electrospray ionization-mass spectroscopy (ESI-MS), capillary electrophoresis (CE), etc. have been used for their detection [25–31]. Unfortunately, these methods are expensive, require

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sophisticated instrumentation and difficult in handling, therefore, are not much of convenience for extensive and continuous use. However, fluorescent probes being highly sensitive with low detection limit provide the best alternate for various traditional methods of biogenic amines and thiols determination. Various reports are available in literature [32–36] for determination of biogenic amines and thiols using various optical techniques. However, there is limited literature on single receptor for estimation of both biothiols and bioamines.

Single molecular probe capable of estimating both analytes will offer huge advantages over single analyte detectors, as the former will employ minimal use of sensing system and can act as differential sensor. Most of the detection methods are based on covalent interaction between the fluorophore and biothiols/biogenic amines [36,37], features long response times with less work in aqueous medium. However, for real-time detection, non-covalent interaction may be more effective in these sensing platforms. Owing to the strong affinity of biothiols and biogenic amines for metal ions [38,39], their additions to a system containing fluorophore-metal complex may induce a change in the binding interaction of metal ion within complex. This interaction may release free fluorophore forming metal-biomolecule complex [40] or biomolecule may simultaneously bind with metal ion and other suitable binding sites on receptor posing changes in fluorescence spectra.

In present work, the tripods with multiple donor atoms tend to organize and engulf around the analyte. These flexible podands provides abundant coordination modes than rigid ones. These podands can adopt different conformations to complement the geometric needs of different metal ions [41], heterocyclic moiety affects the transfer of electron between donor and acceptor group [42]. The naphthyl unit of receptor **1** is the fluorophore responsible for producing detectable signal changes upon guest binding. Further for real application as sensor, receptor **1** is subjected to water soluble FONs (**N1**) and its complex with metal (**N1.Fe^{3+}**) using re-precipitation technique. The fluorescent organic nanoparticles of the complex formed are employed for estimation of various biogenic amines and biothiols and found it selective for tyramine and 4,6-diamino-2-mercaptopyrimidine determination, with a detection limit in low nanomolar range.

2. Experimental

2.1. General information

All chemicals used were of analytical grade and were purchased from Sigma-Aldrich Co. ^{1}H and ^{13}C NMR spectra were recorded on Avance-II (Bruker) instrument, which operated at 400 MHz for ^{1}H NMR and 400 MHz for ^{13}C NMR (chemical shifts are expressed in ppm). The fluorescence measurements were performed on a Shimadzu RF-5301 Fluorescence spectrophotometer. The particle size of nano-aggregates was determined with Dynamic Light Scattering (DLS) using external probe feature of Metrohm Microtrac Ultra Nanotrac Particle Size Analyzer. SEM-EDAX analysis was obtained from JEOL-JSM 6610 LV operating on 15 KV voltage and samples were prepared on carbon tape.

2.2. Synthesis of receptor *1*

(3-Dimethylamino)-1-propylamine (763 μL, 6 mmol) was added to the solution of 2-thiophenecarboxaldehyde (673 μL, 6 mmol) in 12 mL of MeOH and stirred at room temperature for overnight. The liquid product (Schiff's base) thus obtained was reduced using $NaBH_4$ (0.912 g, 24 mmol) and diluted with 5 mL MeOH. The reaction mixture was kept overnight for stirring at room temperature and removed the solvent under reduced pressure. The residue obtained was extracted using $CHCl_3$ and water mixture. The organic layers was extracted and dried over Na_2SO_4 and concentrated to acquire reduced amine. Further, reduced compound was refluxed with 1-naphthyl isothiocyanate (500 mg, 2.7 mmol) taken in $CHCl_3$ (13 mL) at 70 °C and kept for overnight stirring. The excess solvent was removed under reduced pressure and residue was left for evaporation at room temperature. Finally, the obtained compound was washed with diethyl ether which yielded the desired product **1**, having brown colour with yield of 84% and melting point of 138–140 °C. IR (KBr, cm^{-1}) 779 (m), 1155 (s), 1331 (m), 1435 (m), 1470 (v), 1565 (m), 1597 (m), 2916 (s), 3144 (m), 3430 (br m) (Fig. S9). ^{1}H NMR ($CDCl_3$, 400 MHz): 1.88 (q, 2H, CH_2), 2.16 (s, 6H, CH_3), 2.46 (t, 2H, CH_2), 3.82 (t, 2H, CH_2), 5.38 (s, 2H, CH_2), 6.97 (t, 1H, ArH), 7.13 (d, 1H, ArH), 7.27 (d, 1H, ArH), 7.46–7.49 (m, 4H, ArH), 7.76 (t, 1H, ArH),7.85 (m, 2H, ArH), 11.14 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz): 24.48, 44.5, 46.16, 49.81, 54.02, 123.68, 125.38, 125.66, 126.10, 126.10, 126.19, 127.10, 127.40, 128.46, 131.15, 134.77, 137.76, 140.34, 185.95 ESI-MS m/z = 384.16 $[M+H]^+$. CHN analysis: expected C: 65.76%, H: 6.57%, N: 10.95%, S: 16.72%, found C: 65.81, H: 6.42; N: 10.76; S: 17.01.

2.3. Synthesis of nano-aggregates

Fluorescent organic nanoparticles (FONs) of **1** were prepared with re-precipitation method such that 0.5 mL of stock solution of **1** was injected slowly to 100 mL of double distilled water followed by sonication for 10 min. The concentration of **1** in 1 mL DMF (1 mM) was found to be appropriate amongst various concentrations of compound **1** dissolved in DMF. Nano-aggregates of FONs and Fe^{3+} complex were prepared by the addition of ferric nitrate salt.

2.4. Recognition studies

Fluorescence spectroscopic technique was used to study the recognition behaviour of FONs of **1**/complex of FONs and measurements were determined at 25 ± 1 °C. The solutions prepared were kept for half an hour to obtain uniformity and shaken well before use. For cation binding behaviour of FONs of **1** (**N1**) in aqueous medium, 25 μM of metal nitrate salts were added to 5 mL solution of N1 and titration with Fe^{3+} were done using different concentration of $Fe(NO_3)_3$ in volumetric flasks. Similar studies were followed on the same pattern for **N1.Fe^{3+}** complex during determination of tyramine. To evaluate binding and interference behaviour of **N1.Fe^{3+}** complex, 150 μM solution of each biogenic amine were used. Competitive experiment was performed for tyramine and 4,6-diamino-2-mercaptopyrimidine estimation by preparing solutions of **N1.Fe^{3+}** with and without other corresponding biogenic amines (100 μM) and biothiols (300 μM). Effect of ionic strength was checked with different concentration of TBA salt of perchlorate (0–100 equiv.) and experiments showing the response time were also carried out by recording the fluorescence spectrum of **N1.Fe^{3+}** complex.

3. Results and discussion

3.1. Synthesis

The receptor **1** was synthesized by the condensation reaction of N,N-dimethyl-1,3-diaminopropane and 2-thiophene carboxaldehyde followed by reduction of Schiff's base with $NaBH_4$ in methanol. The reduced product obtained was reacted with 1-naphthylisothiocyanate to yield final product (Scheme 1). Compound **1** was characterized using techniques like NMR, FTIR spectroscopy and Mass spectrometry (Figs. S1–S3 and S9).