Sensors & Actuators: B. Chemical 270 (2018) 552–561

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Spermine detection from urine and blood serum using ionic self-assembly of benzimidazolium based dipod and dodecylsulfate

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ARTICLE INFO

Keywords:
Ionic self-assembly
Benzimidazolium dipod
Dodecyl sulphate
Spermine
Fluorescence
Biofluid

ABSTRACT

One of the approaches clinically used for the early diagnosis of cancer is based on the detection and quantification of spermine from the urine. Herein, we have developed platforms of ionic self-assemblies **ENS-1** and **ENS-2** which elicit highly selective fluorescence quenching with spermine. **ENS-1** can detect as low as 6 nM spermine from urine and blood serum samples – a significantly lower concentration required for the early diagnosis of cancer. **ENS-1** and **ENS-2** could be generated *in situ* through ionic self-assembly of 1-(2-naphthyl)benzimidazolium based fluorescent molecules **DI-NAP** and **TET-NAP** with sodium dodecylsulfate (SDS) in HEPES buffer (containing 5% DMSO) which shows blue fluorescence centred at 425 nm. The practicability of **ENS-1** for determining spermine from natural samples could be ensured by the stability of its fluorescence intensity over a period of more than a month, over a broad pH range of 5–10 and also its ability to retain fluorescence intensity at 25 °C even after variation of its temperature between 10 and 60 °C. The mechanism of interaction of **ENS-1** and **ENS-2** with spermine, as obtained from fluorescence, UV–vis spectroscopy and DLS studies reveals binding of the spermine on surface of **ENS-1** and **ENS-2** responsible for the observed changes.

1. Introduction

The biogenic aliphatic polyamines viz. spermine, spermidine and putrescine, found in nearly all living cells are responsible for cell growth and are involved in multiple processes viz. protein synthesis, stabilization of nucleic acid conformations, reducing damage due to reactive oxygen, controlling activity of brain glutamate receptors involved in learning and memory etc. [1–5] Though their total intracellular concentration is in millimolar range but remain mostly in bound state to DNA, RNA, proteins, and phospholipids due to their polycationic nature at physiological pH. The concentrations of free polyamines are low in urine or blood of healthy human. However, alteration in their concentrations in urine or blood can be taken as indicators of mall-functioning in the living systems, usually related to the presence of malignant tumours and are regarded as the biomarkers for the early diagnosis of cancer [5–10]. Therefore, the measurement of polyamine excretion levels in physiological fluids can provide a valuable test in the diagnosis of cancer [11] and in monitoring the response to therapy.

Amongst biogenic amines – spermine under physiological conditions exists in tetracationic state and is expected to bind most strongly with anionic species amongst all biogenic amines, owing to strong chelate effect. The traditional methods for spermine determination such as chromatography, mass spectrometry or immunoassays are quite cumbersome, time consuming and require expensive instrumentation [12–16]. In recent years, the fluorescence based procedures have been found to be significantly more sensitive and selective to provide real-time detection of spermine [17–29]. The different types of fluorometric systems viz. small organic molecules [17–20] and their complexes with metal ions [21], conjugated polymers [23,24], nanoparticles [25–28], supramolecular self-assemblies [23,29] have been reported for the quantification of spermine. However, in general, these probes suffer from poor selectivity towards spermine amongst other biogenic aliphatic amines and necessitate the development of new systems for the determination of spermine.

The classical fluorescent receptor approach requires the covalent linkage between the binding sites and the indicator [30,31]. The chemosensing ensemble systems [32–34] (first used by Anslyn and coworkers [35] in 1998) do not require the presence of both the binding site and indicator on the same molecule and remain free from the tedious and complicated design and synthesis of the fluorescent receptor. The formation of chemosensing ensembles depends on the non-covalent

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https://doi.org/10.1016/j.snb.2018.05.050
Received 15 March 2018; Received in revised form 9 May 2018; Accepted 10 May 2018

interactions such as hydrogen bonding, electrostatic, donor-acceptor π-π stacking, van der Waals forces and hydrophilic and hydrophobic interactions between the indicator (fluorescent/chromogenic dye) and the receptor. Such ensembles have an advantage of being obtained *in situ* in aqueous solutions under physiological conditions. If the receptor is able to bind with both the fluorescent dye and the analyte but with different affinity, the addition of the analyte to the fluorescent dye −receptor ensemble results in change in fluorescence properties of the ensemble. Moreover, since assembly of the indicator and the receptor units depends on the non-covalent weak forces, the ensembles can be more sensitive to the analyte over conventional approach. In the case of binding of spermine with chemosensing systems [17–29], the basic mechanism involves the strong interactions of spermine − a tetracationic species under physiological conditions, with the negatively charged sites of the chemosensing system which results in change in the interactions of the negatively charged centres with that of the indicator moiety leading to change in optical properties of the indicator reporting the presence of spermine.

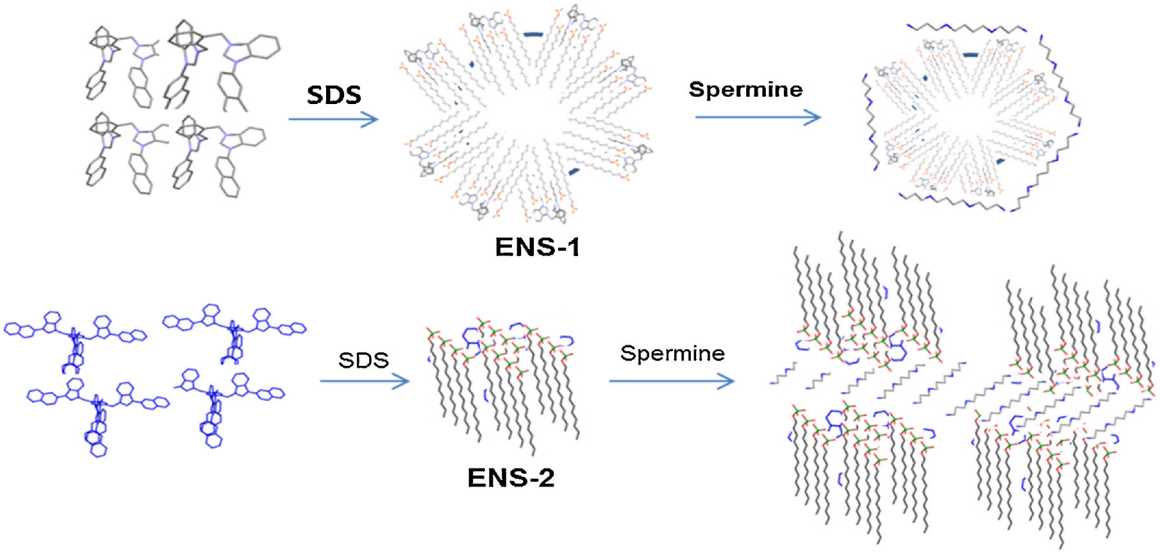
Recently, we have shown that N-arylbenzimidazolium [36–42] and pyridinium [43] derivatives owing to their strong electrostatic and hydrogen bonding interactions with negatively charged species provide numerous possibilities for the selective interactions with anionic species. In continuation of our interest in using fluorogenic ensembles for the determination of biologically relevant species [44], herein we have synthesized 1-(2-naphthyl)benzimidazolium based fluorescent probes **DI-NAP** and **TET-NAP** which in the presence of sodium dodecylsulfate (SDS) form ensembles **DI-NAP–SDS** (referred as **ENS-1**) and **TET-NAP–SDS** (referred as **ENS-2**) in 95% buffer – DMSO solution and are associated with increase in blue fluorescence centred at 425 nm. These ensembles are quite stable towards variation in temperature between 20–60 °C, pH and time duration of over a month. **ENS-1** and **ENS-2** elicit highly selective fluorescence quenching with spermine and can detect as low as 6 nM spermine (using **ENS-1**) from urine and blood serum samples – a significantly lower concentration required for the early diagnosis of cancer from urine and blood samples. **ENS-1** can be used for the naked eye detection of ~100 nM concentrations of spermine under 365 nm light illumination. The mechanism of interaction of **ENS-1** and **ENS-2** with spermine, as obtained from fluorescence, UV–vis and DLS studies reveals that spermine binds on the surface of **ENS-1** and results in decrease in size of the aggregates and in the case of **ENS-2**, spermine leads to aggregation of the **ENS-2** ensembles to large size aggregates (Scheme 1).

2. Experimental section

2.1. General remarks

All reagents were purchased from commercial suppliers (Aldrich, SDFCL, Spectrochem etc.) and used without further purification. 1-(2-Naphthyl)benzimidazole was synthesized by CuI, benzotriazole catalyzed N-arylation of benzimidazole with 2-bromonapthalene as reported in literature [45]. TLC analyses were performed on silica gel plates and column chromatography was carried out over silica gel (100–200 mesh). ^{1}H and ^{13}C NMR spectra were recorded on JEOL 400 MHz FT NMR machine using $CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard. Data are reported as follows: chemical shifts in ppm relative to the tetramethylsilane (TMS) as an internal standard, coupling constants J in Hz; multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet). HRMS spectra were recorded on Brucker MicroToff/QII. The time resolved fluorescence spectra were recorded with ISS Chronos-BH time-resolved fluorescence spectrophotometer. Dynamic light scattering (DLS) measurements were performed at 25.0 ± 0.1 °C using Zetasizer Nano ZS provided by Malvern Instrument Ltd. The deionized water was obtained from ULTRA UV/UF Rions Lab Water System Ultra 370 series and was used for preparing all solutions. UV–vis studies were carried out on Shimadzu UV-2450 machine using slit width of 1.0 nm and matched quartz cells, thermo stated at 25.0 ± 0.1 °C. The fluorescence experiments were performed on ISS Chronos-BH fluorescence spectrophotometer with a quartz cuvette of path length 1 cm. All absorption and fluorescence scans were saved as ASCII files and were further processed in Microsoft Excel™ to produce all graphs shown.

The stock solutions of **DI-NAP** and **TET-NAP** (1 mM) were prepared in DMSO. For experiments 50 μL of stock solution was diluted with 450 μL of DMSO and was diluted with HEPES buffer (0.05 M, pH 7.4) up to 10 mL mark. 3 mL of this solution was taken in quartz cuvette for UV–vis and fluorescence studies. Stock solutions (0.1 M) of sodium salts of inorganic anions viz. OH^-, F^-, Cl^-, Br^-, I^-, NO_3^-, HSO_4^-, ClO_4^-, CN^-, HPO_4^-, $H_2PO_4^-$, ACO^- and organic anions viz. butyrate (C4), hexanoate (C6), octanoate (C8), decanoate (C10), benzoate, decylsulfate, dodecylsulfate (SDS) and dodecylbenzenesulfonate (SDBS) etc and polyamines viz. lysine, phenyl alanine, arginine, pyrophosphate, melamine, 1,3-diaminopropane, diethylenetriamine, hexamethyl-tetramine, oleylamine, octyl amine, protamine, spermidine, spermine were prepared in deionized millipore water and were diluted as required.



Scheme 1. Schematic presentation of formation of **ENS-1** and **ENS-2** and their interactions with spermine.