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Isatin-3-thiosemicarbazone as Chromogenic Sensor for the Selective Detection of Fluoride Anion

Dedicated to 68th birthday of Prof. Dr. Klaus Jurkschat<https://doi.org/10.1515/hc-2020-0104>

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Abstract: In this study, we describe the anion recognition ability of isatin-3-thiosemicarbazone **2**, which contains two different anion recognition units *i.e.* isatin NH and the thiourea moiety. Both have the ability to act as proton donors. Most importantly, a significant colour change of **2** was observed (from light yellow to reddish orange) in organic medium only after the addition of the F[−] anion. No such colour change could be observed for any other anions including Cl[−], Br[−], I[−], H₂PO₄[−], NO₂[−], PF₆[−] etc. The UV-Vis spectroscopic studies also indicate the potential of this compound for selective detection of fluoride anions. ¹H-NMR titrations clearly indicate the formation of the 2.F[−] anionic complex. The Density-functional theory (DFT) calculations are also performed to get further insights on the formation of 2.F[−] complex.

Keywords: Isatin, thiosemicarbazone, chromogenic sensor, fluoride ions, UV-Vis titrations, detection, DFT calculation

Introduction

Anions play an important role in biological systems, as these are the main components in maintaining the

homeostasis of the body [1-3]. But their extensive use may cause serious health issues [4, 5]. That's why, in recent years, their detection became a very important prospective for chemists and biologists and many techniques have been developed for this purpose [6, 7]. Compared to the chemistry of cations, the development of artificial receptors for anion recognition has been much slower, most probably due to the challenges associated with the larger ionic radii, higher solvation energies and diverse topologies of anions [3, 8]. Among the anions, the fluoride ion (F[−]) is of particular interest owing to its established role in dental care and clinical treatment for osteoporosis [9]. An acute intake of a large dose or chronic ingestion of lower doses of F[−] can result in gastric and kidney disorders, dental and skeletal fluorosis, urolithiasis in humans, and even death [10-12]. For these reasons, an improved method for the detection and sensing of F[−] with high selectivity is of current interest in the chemosensor research field. Consequently a variety of artificial sensors have been developed [13, 14]. Due to the ease of operation, high selectivity and low cost the receptors that show absorbance changes (colour change) upon binding with F[−] have been studied extensively [15-18]. Among various chromogenic artificial receptors, the compounds having polarized NH groups as anion binding motifs that preferably show visible colour changes upon binding to anions have attracted considerable attention [19, 20]. These receptors mostly consist of an H-bond donor connected with the chromophore. Thiourea, urea, amide, amine, pyrrole and alcohol groups are frequently used as the as the hydrogen bond donors. On the other hand, most of the organic dyes such as nitrobenzene, indoaniline, anthraquinone and azobenzene or comprehensively conjugated aromatic substances such as porphyrin, oxadiazole and quinoxaline are used as a chromophore in such types of chromogenic anion sensors [21].

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Among these artificial chromogenic chemosensors those based on the thiourea moiety have attracted considerable attention due to the acidic *NH* group that shows strong binding affinity for anions [22, 23]. Unfortunately the simple thiourea moiety cannot act as the chromogenic sensor, therefore some chromogenic groups are always needed to get the quantitative information regarding anion binding [23]. In this context, many bulky optical sensors having thiourea as the binding site have been reported. However, the synthesis of such sensors/receptors require laborious synthetic procedures [22]. Consequently, thiourea linked small sensors/receptors are gaining more appreciation [8], although the fabrication of simple and small anion sensor/receptors having appropriate sensitivity and selectivity is still challenging.

As a continuation of our recent interests in supramolecular chemistry [24-29], herein we report a simple artificial colored receptor based on the well-known color dye isatin. The proposed receptor owns two different types of *NH*-hydrogen bond donors (*NH*-isatin and *NH*-thiourea-). Isatin not only provides extra *H*-bonding site but also acts as the signaling unit by shifting the absorption to the Vis-region of the electromagnetic spectrum.

Results and Discussion

The synthesis of targeted receptor **2** was carried out according to the reported method [30]. As the synthesised receptor is a coloured compound, therefore colorimetric

studies were carried out to check its ability to act as naked eye sensors for the anions *via* observing the color changes. This method of sensing is highly appreciated due to the low cost and easy detection without the help of any instrument. Interestingly, the addition of F^- anion ($1\ \mu\text{M}$, 2.5 ml, acetonitrile) to a 10 mM solution of receptor **2** (2.5 ml, acetonitrile) resulted in a notable colour change from light yellow to reddish orange, clearly visible to naked eye (Figure 1). In contrast, the addition of Br^- , I^- , F^- , Cl^- , AcO^- , $H_2PO_4^-$, ClO_4^- , PF_6^- , NO_2^{2-} and SCN^- resulted in no visible changes in color (Figure 2).

The selective color change of receptor **2** upon addition of Fluoride ions, motivated us to conduct further experiments. For this purpose, we have conducted UV-Vis absorption spectra of receptor **2** in acetonitrile. The absorption spectrum of receptor **2** in acetonitrile solution dominated by two peaks; one present at 265 and other at 370 nm (Figure 3). Upon the addition of appropriate volume of 1 equivalent of various anions, as expected;

Figure 1 Structure of targeted receptor **2**

Figure 2 Changes in colour of **2** (10 mM) upon addition of different anions ($1\ \mu\text{M}$), A: (Blank), B: (Br^-), C: (I^-), D: (F^-), E: (Cl^-), F: (AcO^-), G: ($H_2PO_4^-$), H: (ClO_4^-), I: (PF_6^-), J: (NO_2^{2-}), K: (SCN^-)

the F^- selectively caused the appearance of the new band at 470 nm, and a decrease in the band at 370 nm with an isosbestic point at 410 nm. However, no significant change was observed upon addition of bromide, chloride, iodide, acetate, phosphate etc. in the solution of **2** (Figure 4). This ratiometric behavior of the receptor towards the Fluoride ions clearly suggest formation of **2**· F^- anion complex. A bathochromic shift of about 100 nm may be attributed due to the interaction of fluoride anion with **2** which causes hydrogen-bond or negative charge (in case of complete deportation)-induced electron delocalization.

After having discovered that receptor **2** can recognize fluoride anions preferably over the other anions, titration studies were performed to check the quantitative behavior of these receptors towards different concentrations of fluoride anion. It was observed that by gradually increasing the concentration of the fluoride anion,

Figure 3 Absorbance spectrum of **2** (1 μ M in acetonitrile)

Figure 4 The changes observed in the absorbance profile of **2** (10 mM) upon addition of 1 μ M solutions of different anions

the intensity of absorption band at 370 nm of **2** decreased with a blue shift of 10 nm and intensity of absorption band at 470 nm increased (Figure 5), which suggest the formation of the new species with more extended conjugation. The isosbestic point at 410 nm indicates that two species are in equilibrium throughout the titration process. After the addition of 2 equivalents of Fluoride ions no obvious change was observed in absorption of the receptor. This suggests that the equilibrium shifted towards left side either due to the complete deportation of the receptor or due to the formation of the stable anionic complex. The receptor shows a linear relationship with added Fluoride ions over wide range (0–2.0 μ M) with a detection limit of 2.37 μ M (Figure 6) [31].

Figure 5 UV-Vis absorption spectra of receptor **2** (1 mM) in acetonitrile upon addition of 0–2.0 μ M of tetrabutylammonium fluoride (TBAF)

Figure 6 Benesi-Hildebrand (Linear regression) graph between concentration of fluoride (0–2 μ M) added and of relative absorbance intensity (A_{486} nm/ A_{353} nm) of **2**

From the spectroscopic studies it is difficult to predict the actual phenomenon for the selective colour change of the receptor in the presence of fluoride ions. To affirm it a real phenomenon we have carried out ^1H NMR titration experiments with receptor molecule in $\text{DMSO}-d_6$ by stepwise addition of equivalents of F^- as the $[\text{Bu}_4\text{N}]\text{F}$ salt. As receptor **2**, has three acidic Hydrogens, two from the thiourea moiety (labelled as *a* and *b*) appeared at 10.89 and 11.35 ppm and the third proton from the N-H of the isatin (labelled as *c*) appeared at 12.62 ppm (Figure 7). The addition of 0.5 equivalent of fluoride ions caused the broadening and the slight shifting of the all three acidic protons. The broadening of two thiourea protons are more obvious than the isatin protons. This suggests that initially the fluoride ions interact with thiourea protons because compared to the isatin proton they are more acidic. When 1 equivalent of fluoride ions was added the broadening of the isatin N-H proton was observed which clearly suggests the formation of the **2.F⁻** anionic complex. This anionic complex forces the tautomerization of isatin into an Amide-Iminol tautomer [32] which results in the colour change, the other anions used in this study have not enough basic character to trigger the isomerization (Figure 8).

To gain an insight into the electronic structure, photophysical properties of receptor **2** and receptor **2.F⁻** adduct, DFT-B3LYP/6-311G, level calculations were performed using Gaussian 09 software. Figure 9 shows the optimized structures, isodensity surface plots (graphical representations) of HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) of receptor **2** and receptor **2.F⁻** adduct. Figure 9 also demonstrates the values of energy levels and band gaps. The LUMO energy level (-2.49 eV) of receptor **2.F⁻** adduct is found to be lower than that of the receptor **2** (-2.16 eV) and the HOMO energy level (-5.15 eV) of receptor **2.F⁻** adduct is observed to be higher than that of the receptor **2** (-5.49 eV); therefore, the band gap between the HOMO and LUMO of the receptor **2.F⁻** adduct (2.66 eV) is smaller than that of receptor **2** (3.33 eV). According to Planck-Einstein relation, band gaps are in good agreement with the experimentally observed *bathochromic* shift in the absorption profile of **2** (1 μM) from 370 nm to 470 nm upon addition of 1 μM solutions of F^- anions [33, 34].

In the case of **2**, HOMO is localized on the hydrazinecarbothioamide moiety while LUMO is localized throughout the whole molecule except the fluorobenzene entity. However, in the case of receptor **2.F⁻** adduct,

Figure 7 Change in ^1H -NMR spectrum of **2** with gradual addition of TBAF

Figure 8 Purposed mechanism for the sensing of Fluoride ion

Figure 9 Frontier molecular orbitals of receptor 2 and receptor 2.F⁻ evaluated from the DFT calculations using Gaussian 09 software

twisting in molecular framework with spatial separation of frontier molecular orbitals (FMOs) is observed. Hence the fluoride ion attachment in the receptor leads to a dramatic change in the FMOs as well as molecular shape resulting in the observed change in absorbance.

Conclusion

In conclusion, we have synthesized and characterized isatin-3-thiosemicarbazone as a potential anion sensor that allows naked-eye detection of fluoride anions over other anions in acetonitrile solution. We have also performed UV-Vis titrations to validate selective sensing of fluoride anions in the presence of various other anions. The receptor was highly selective towards

fluoride anions because the receptor perceived no interference from any of other interfering anions. We have also discussed the complete mechanism for the selective sensing of fluoride ions.

Experimental

Gallenkamp melting apparatus was used to determine melting point of the synthesized compounds. The IR spectral data of synthesized solid substances was recorded on Bio-Rad Merlin FTIR spectrophotometer as KBr discs and neat liquid. BurkerAvance spectrometer was used to record ¹H NMR and ¹³C NMR. This spectrophotometer was operated at 300 MHz for ¹H NMR and 75 MHz for ¹³C NMR. Spectra were recorded in DMSO-*d*₆ solvent and TMS was

used as internal standard. Chemical shift values were expressed in ppm. Singlet, doublet, triplet and multiplet were abbreviated as *s*, *d*, *t*, *m* respectively. Coupling constants were calculated in Hz. Absorption spectra of compounds were recorded in Pharmacy UV-1800 UV-Visible Spectrophotometer Shimadzu (Japan). Quartz cells having a path length of 1 cm were used for running the sample.

General procedure for the synthesis of isatin-3-thiosemicarbazones (2)

5-chloro isatin (1.3g, 7.17mmol) was added drop wise to a stirred hot solution of respective thiosemicarbazide (1.3g, 6.39 mmol) in the ethanol: water (15:5 mL), and then heated at reflux for 5 h. After complete conversion of starting materials (monitored through TLC), the reaction mixture was cooled to room temperature, which resulted in the formation of precipitates. Precipitates were filtered off and washed with ice cold ethanol and dried in vacuum oven. Light yellow orange; Yield: 80%; R_f value: 0.3 (ethyl acetate: *n*-Hexane); m.p.:258 °C-260 °C; IR (pure, cm⁻¹):1687.84 (C=O), 3353.01 (NH), 3327.77 (NH), 3213.55 (NH), 825.32 (C=S), 1611.66 (aromatic C=C);¹H-NMR (DMSO-*d*₆, 300MHz, chemical shift value): 6.94 (1H, d, J=8.4 Hz), 7.39-7.43 (3H, m), 10.93 (1H, s), 11.37 (1H, s), 12.37 (1H,s), 7.636 (1H, d, J=10.5), 7.46-7.50 (1H, m), 7.89 (1H, d, J=2.1) PPM; ¹³C-NMR (DMSO-*d*₆, 75MHz): 163.05 (C-F), 178.78 (C=S), 162.86 (C=O), 141.67 (C=N), 121.33, 127.11, 123.33 (C isatin).

Recognition studies

The sensing/recognition studies were carried out at a temperature of 25 ± 1 °C. The stock solution (10 mM) of **2** was prepared in acetonitrile. The possible influence of the anions on receptor was studied by adding the appropriate volume of tetrabutylammonium salts of anions (1 μM) to the stock solution of **2** and color change was observed with the naked eye and the UV-Vis spectra were recorded without any delay. To investigate the effect of increase in concentration of fluoride anion on **2**, titration studies were performed. Different concentration of solutions (0.2, 0.4, 0.6, 0.8, 1.2, 1.6 and 2.0 μM) of fluoride ion were added to volumetric flasks that already contained chromogenic chemosensor **2** (10 mM). The effect of increasing the anion concentration on the absorption spectra was recorded by UV spectrometer after gradual addition of different concentration solutions of fluoride ion. Selectivity of

chemosensors towards F⁻ was studied by adding the solution (1 μM) of different anion salts into the mixture of chemosensors and F⁻ solution followed by recording the absorption profile of chromogenic chemosensors **2**. ¹H NMR titration experiment was carried out in DMSO-*d*₆ by stepwise addition of equivalents of F⁻ as their [Bu₄N] F salt to the known concentration (1 mM) of the receptor solution.

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