Research Article

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Isatin as a simple, highly selective and sensitive colorimetric sensor for fluoride anion

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Abstract: Herein, we report the fluoride anion sensing properties of a commercially available and inexpensive organic compound, isatin, which is found to be a highly selective and sensitive sensor. In naked-eye experiments, by addition of fluoride anions, isatin shows a dramatic color change from pale yellow to violet at room temperature, while the addition of other anions, *i.e.* Cl^- , Br^- , I^- , ClO_4^- , H_2PO_4^- and PF_6^- , did not induce any colour change. Additionally, recognition and titration studies have also been done through UV/Vis spectroscopy. Isatin displayed a new absorption band at 533 nm after the addition of fluoride anions, which is presumably due to acid-base interaction between isatin and fluoride anions, while other anions did not trigger noticeable spectral changes. The detection limit was observed to be 0.367 ppm. DFT calculations were also performed to further explain the behavior of receptor 1 towards the F^- anion. Owing to high sensitivity and selectivity, isatin can be useful in the detection of biologically or environmentally important fluoride anions at very low concentration.

Keywords: Isatin, chromogenic sensor, ion detection; fluoride, UV-Vis titrations, DFT calculations

Introduction

In our everyday life, anions are valuable because they regulate and/or are responsible for numerous environmental and biological processes [1-16]. For instance, anions are involved in the production of electrical signals, controlling osmotic pressure, activating signal

transduction pathways, maintaining cell volume, *etc* [17]. Therefore, the changes in anion flux across cell membranes is increasingly being identified as the foremost cause of various disorders including osteoporosis [18], Pendred's syndrome [19], Dent's disease [20], Bartter's syndrome [21] and cystic fibrosis [22]. Further, anions are important for many industrial processes and are often found as harmful pollutants [23]. Also, anions are present in almost 70% of all active sites of enzyme, which play a significant part in genetic information storage [24]. Moreover, anions play crucial roles in the fields of catalysis and medicine, although pollutant anions have been mainly linked to carcinogenesis (metabolites of benzoate and acetate) [25], eutrophication of rivers (from the application of phosphate-based fertilizers) [26] and contamination of drinking water (by fluoride pollution) [27].

Among anions, fluoride is the most important, owing to its involvement in numerous environmental and biological processes [28-31]. Fluoride is known to have a hard Lewis base nature, the smallest ionic radius, and the highest charge density of any anion [32]. Naturally, fluoride ions occur in the sea, in soils and rocks, and in groundwater supplies. Numerous studies have shown that fluoride in drinking water or toothpaste can help with dental health [33, 34]. Also, fluoride can help increase bone mass and hence has the potential for treating osteoporosis [35, 36]. Because of these benefits, water fluoridation had become widely used by 1960 [37]. However, later studies have shown that overexposure to fluoride can be detrimental, especially to the teeth [38], brain [39], kidney [40] and bone [41], resulting in a disease known as fluorosis. This diversity of function of the fluoride anion, both the benefits and perniciousness, makes its detection of considerable interest. In this context, organic colorimetric chemosensors are of particular importance due to their simplicity [29, 42-46]. Color changes visible to the naked eye are preliminarily used as signals for detection of anions without any equipment being required. However, the synthesis of such chemosensors usually requires expensive reagents, and long or tedious synthetic processes, making them less applicable. Therefore, the development of a cheap, non-corrosive, environment friendly, safe, selective, and sensitive chemosensor remains attractive [42, 43].

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Isatin (1*H*-indole-2,3-dione) **1** (Figure 1) is a commercially available and inexpensive organic compound that was first discovered by Erdman and Laurent in 1840 as an oxidation product of indigo [47]. Almost 140 years after its discovery, it was identified as a natural product present in plants, animals, fungi, symbiotic bacteria and marine molluscs [47]. Isatin **1** is also considered important in many physiological pathways [48-50]. Apart from its applications in medicinal chemistry [47], it has been extensively employed as a signaling unit in various receptors [51, 52] and some of its derivatives have also been investigated as chemosensors for anion detection [53, 54]. Careful analysis of its features reveals that isatin **1** has an acidic NH group and intense color which are prime requirements for a chemosensor [29, 42-46]. The interesting structure combined with our recent interest in isatin-derived compounds [55-59] and in developing chemosensors [6, 60] motivated us to investigate the behavior of commercially available isatin as an anion sensor. Fascinatingly, as discussed below, isatin is found to be a highly selective and sensitive chemosensor for fluoride anions in the presence of various other anions, as its acidity allows it to interact only with fluoride anions. To the best of our knowledge, this is one of the cheapest chemosensors reported so far for the selective recognition/sensing of the fluoride anion.

Figure 1 Structure of isatin.

Results and discussion

As isatin **1** is a colored compound, its potential in anion recognition/sensing was first monitored by naked eye experiments. This method of sensing is highly valued due to its low cost and easy detection without the help of any instrument or equipment [29, 42-46]. Interestingly, the addition of one equivalent of F^- anion to the solution of isatin **1** resulted in a dramatic color change from pale yellow to violet, clearly visible to the naked eye. Most importantly, the color change was observed even at a low concentration of fluoride anion (10 mM). In contrast, the addition of other anions resulted in no visible changes in color (Figure 2).

After obtaining the preliminary information of selectivity for fluoride anion with naked eye experiments (Figure 2), the chemosensor **1** was further evaluated by UV-Vis spectroscopy. Absorption spectra were recorded after the addition of one equivalent of different anions to the solution (0.01 μ M) of isatin **1** in MeCN to observe the selectivity pattern. All the solutions were well shaken to ensure the homogeneity of the solutions before recording the absorption spectrum. Figure 3 shows the changes in the UV-Vis spectra of **1** upon the addition of F^- , Cl^- , Br^- , I^- , ClO_4^- , $H_2PO_4^-$ and PF_6^- (as their *tetra-*n**-butylammonium salts). As shown in the Figure 2, **1**, which absorbs at 410 nm in MeCN solvent, demonstrated a new and bathochromic shifted absorption band at 533 nm after the addition of fluoride anion. A bathochromic shift of more than 100 nm may be attributed to the hydrogen bonding/acid-base interaction between **1** and the fluoride anion. In other words, the interaction of fluoride anion with **1** caused hydrogen-bond or negative charge (in case of complete deprotonation)-induced electron delocalization. Under similar conditions, no changes in the absorption spectra could be observed upon addition of other anions, i.e. Cl^- ,

Figure 2 Color changes of the chemosensor **1** (0.01 μ M) in MeCN with the addition of 1 equivalent tetrabutylammonium salts (from left to right: **1**, F^- , Cl^- , Br^- , I^- , ClO_4^- , $H_2PO_4^-$ and PF_6^-).

Br^- , I^- , ClO_4^- , H_2PO_4^- and PF_6^- . These results clearly indicate the ability of **1** for selective fluoride ion recognition in the presence of various other anions (Figure 3).

After ascertaining that compound **1** can recognize fluoride anions preferentially over other anions, titration studies were performed to check the quantitative behavior of this chemosensor towards different concentration of fluoride anion (Figure 4). In these studies absorption spectra were recorded for each concentration of fluoride anion. It was observed that by gradually increasing the concentration of fluoride anion, the intensity of the absorption band of **1** at 410 nm decreased and the intensity of the absorption band at 533 nm increased with a sharp isobestic point development at 470 nm, indicating the existence of only two species in equilibrium throughout the titration process (Figure 5). From this, it may be

deduced that the intermolecular interaction between **1** and the fluoride anion results in complete deprotonation rather than hydrogen bonding, owing to its greater influence on the electron density of the isatin nucleus.

The binding interactions of **1** with the analyte was further examined via Job's plot analysis. As shown in Figure 6, the gradual increase in the intensity of the absorption band of **1** with the increase in fluoride anion concentration showed a linear relationship up to one equivalent of fluoride anion, signifying a 1:1 binding ratio between **1** and the F^- ion. Moreover, according to the UV-Vis titration experiment the chemosensor **1** showed a detection limit [61] of 0.367 ppm with $R^2 = 0.9654$.

Computational studies

The observed selectivity and sensitivity of chemosensor **1** towards the F^- anion can be explained on the basis of density functional theory (DFT) calculations. The optimized structures of **1** and its anion in the ground state were obtained from DFT/B3LYP/6-31G using the *Gaussian 09* software package. Figure 7 shows the optimized structures, graphical representations (isodensity surface plots) of the LUMO (lowest unoccupied molecular orbital) and HOMO (highest occupied molecular orbital) of **1** and its anion. Figures 7 also displays the band gaps and energy

Figure 3 Absorption spectral changes of chemosensor **1** (0.01 μM) upon addition of 1 μM solutions of different anions.

Figure 4 UV-Vis titrations of **1** (0.01 μM) with different concentrations of TBAF in MeCN.

Figure 5 Proposed mechanism of detection of **1**.

Figure 6 Linear regression graph between added concentration of TBAF (0-2 $\times 10^{-8}$ M) and of relative absorbance (at 410 and 533 nm) of chemosensor **1**.

Figure 7 Frontier molecular orbitals of chemosensor 1 and its anion.

level values. The HOMO energy level (-5.16 eV) of the isatin anion is higher than that of the chemosensor 1 (-5.54 eV) and the LUMO energy level (-2.79 eV) of the isatin anion is lower than that of the chemosensor 1 (-2.50 eV); hence, the band gap between the LUMO and HOMO of the isatin anion (2.37 eV) is smaller than that of the sensor 1 (3.04 eV), which is in good agreement with the bathochromic shift in absorption ($\lambda_{\text{max}} = 533 \text{ nm}$) detected upon treatment of chemosensor 1 with F^- anions. It is noted that the relation between the energy band gap and absorption spectra was estimated from the Planck-Einstein relation [62].

In the case of 1, both the HOMO and LUMO were localized throughout the whole molecule. However, in the case of the anion of 1, spatial separation of FMOs is noticeable, i.e., both HOMO and LUMO were chiefly localized on the electron-rich pyrrolidine ring. This orbital distribution revealed that the deprotonation of 1 should impact the LUMO and HOMO evenly and therefore constitute the basis of an optical response of 1 to addition of F^- (Figure 7).

Conclusions

In summary, a commercially available colored organic compound, isatin, was investigated for selective fluoride anion sensing using UV/Vis spectroscopy and naked-eye experiments. The results reveals that isatin is a novel colorimetric chemosensor for selective detection of fluoride ions in acetonitrile. Isatin shows changes in both its color and its UV-Vis absorption spectra upon the addition of fluoride, while the other anions, such as Cl^- , Br^- , I^- , ClO_4^- , H_2PO_4^- and PF_6^- , did not produce any noticeable change. The selectivity of the isatin receptor towards the fluoride anion over the other anions is attributed to the small size and greater charge

density, hence Lewis basicity, of the fluoride anion, which led to the complete deprotonation of the receptors. However, the other anions/Lewis bases were not strong enough to deprotonate the receptors. The results further demonstrated that the isatin receptor respond in microseconds and in linear fashion over a wide range of fluoride concentrations and a very low concentration of fluoride anion could be detected, i.e., the limit of detection of isatin is 0.367 ppm. Owing to its solubility in water as well as in organic solvents, isatin can be useful in the detection of biologically or environmentally important fluoride anions in both aqueous and organic media. The high selectivity and sensitivity of isatin towards fluoride anions makes this molecule highly attractive and this study will provide an ideal platform to explore this molecule further in the field of anion sensing/recognition.

Experimental

Naked-eye detection studies

For naked eye experiments, 1 (0.01 μM) 3 ml was placed into a glass vial and 1 equivalent of each anion was added to the glass vial.

Recognition studies

The UV-Vis spectra were recorded using a BioMate 3S UV-Visible Spectrophotometer (Thermo Scientific). All absorbance assays were performed in 100 μL quartz cuvettes. The recognition studies were performed at 25°C. 2 μM solutions of 1 in acetonitrile were prepared and 2.5 mL of each was taken in volumetric flasks. The binding behavior of 1 was studied by adding 2.5 mL solutions (4 μM)

of different anions (TBAF, TBACl, TBABr, TBAI, TBAPF₆, TBAH₂PO₄ and TBAClO₄) into the solutions of 1 and the absorption spectrum of each solution was recorded.

Titration studies

For titration studies, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 2.0 equivalents of F[−] were individually added to volumetric flasks containing 1 and absorption spectra were recorded for set after each aliquot addition of F[−].

Selectivity studies

Possible interference due to different anions was studied by adding one equivalent of different anions to 1 μM solution of 1. Absorption spectra were recorded and no interference was found.

Sensitivity studies

The sensitivity of 1 towards fluoride anion was checked by adding a solution of F[−] (μM) to a solution of 1 (0.01 μM) and absorption spectra were recorded as a function of time at short intervals.

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