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ARTICLE

Development of an AIE based fluorescent probe for the detection of nitrate anion in aqueous solution over a wide pH range

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A new type of water-soluble anion fluorescent probe based on ionic interaction-triggered AIE is reported. The positively-charged probe displayed highly selective sensing and imaging of NO_3^- anion over other environmentally and biologically relevant species through a significant AIE turn-on fluorescence signal in aqueous solution over wide pH range. Hydrophobic aggregation tendency of the probe itself in aqueous solution was supposed to beneficial for the target anion to overcome the high dielectric constant of water. As a result, ionic interaction between the positively-charged probe and the negative NO_3^- anion occurred in aqueous media and thereby AIE-based selective turn-on fluorescent signal was achieved. These results demonstrate that the ionic interaction triggered AIE can be designed as new sensing mechanism for detection of anions in aqueous media and living cells.

Introduction

Given the biological, environmental and chemical importance of anions, receptors for anion recognition, sensing and transport are important.[1] Compared with isoelectronic cations, anions are larger and have lower charge-to-size ratios with various geometrical shapes.[2] Receptors that rely on hydrogen bonding, ion pairs, the hydrophobic effect, and electrostatic or π-stacking interactions as anion recognition binding sites are being developed.[1-3] Currently, considerable attention is being paid to fluorescent probes for the detection of anions attributed to their excellent advantages over other methods,[4] such as high sensitivity, operational easily, and potential for bioimaging analytes in vivo and living cells.[5] Although advances in the development of such new fluorescent probes, there are still some limitations in quantitative detection and bioimaging. For example, from the viewpoint of practical applicability, a fluorescent reagent for the imaging of anions must meet various requirements, including cell permeability, water solubility, nontoxicity and so on. However, due to the intrinsic hydrophobic effect of most fluoroionophores, such probes can be only utilized in organic solvents. In particular, interference from other anions and hydrogen-bonding interactions with water molecules seriously hinder the development of anion chemosensors and their further applications.

In 2001, Tang and co-works reported that fluorophore with restricted intramolecular rotations were non- or weakly fluorescent in dilute solutions, but emitted strong fluorescence upon aggregation, which was thus called aggregation-induced emission (AIE).[6] Then, AIE has been used as a new mechanism in probe design. It relies on the aggregation of probe monomers with different formulations and surface functionalities induced by analytes through noncovalent interactions.[7] The obtained AIE entities usually exhibit unique spectroscopic properties compared with the constituent monomer. As a result, the turn-on fluorescence feature of the molecular AIE bioprobes allows direct visualization of specific analytes and biological processes, in particular in aqueous media, with higher sensitivity and better accuracy than traditional fluorescence probes.[8] However, although this new sensing mechanism has been widely employed to construct fluorescent probes for cations and biological molecules, reports on anions are rare.

Among the various AIE luminogens, those based on an anthracene core adopt nonplanar conformations and propeller-like shapes, and the steric effect of the bulky aryl rings may induce conformational twists, giving rise to marked AIE effects.[9] For example, Tian and co-workers reported a series of pioneering studies on divinylanthracene derivatives with fascinating properties.[10] In particular, when pyridyl groups were introduced into the molecular structure, interesting phenomena were observed. Such as aggregation-induced tunable emission colour changes from green to red and wavelengths spanning the visible region under different external stimuli.[10a] Single-crystal X-ray structures indicated that such remarkable fluorescence changes can be attributed to different π–π interaction between adjacent anthracene moieties.[10a] This result suggested that the anthracene group should be an ideal candidate for constructing turn-on fluorescence probes based on the AIE effect. On the other hand, ionic interaction between oppositely charged species, is one of the strongest noncovalent interactions, and has been

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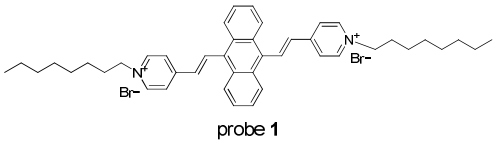
widely used in electrostatic self-assembly in the solid state[11] and ionic self-assembly in solution.[12] Although the wide availability of charged species and the simplicity operation allow the ionic interactions to be utilized in preparation of various functional materials, it remains a great challenge to exploit this kind of interaction to develop the related materials in aqueous solution. This is mainly because of the high dielectric constant of water (ε = 78.5, 298 K)[13] and hence electrostatic interactions become significantly weakened in aqueous media. Recently, we have successfully prepared a water-soluble fluorescent probe containing a phenylenevinylene pyridinium cation motif, which showed highly selective sensing ability towards NO_3^- anion in acidic aqueous media through ionic interaction.[14] In that system, the hydrophobic nature of the fluorophore moiety and aliphatic chains in aqueous solution played an important role in the recognition procedure of the target anions. In other words, the hydrophobic aggregation tendency of the probe itself in aqueous solution was beneficial for the NO_3^- anion to overcome the high dielectric constant of water and engage in ionic self-assembly. However, the recognition behaviours should be carried out in acidic condition. With these observations in mind, we have now synthesized a new AIE fluorescent probe based on a divinylanthracene core. This probe can serve as a turn-on fluorescence chemosensor for NO_3^- anion in acidic, neutral and basic aqueous solutions through ionic interaction-induced aggregation.

Results and discussion

As shown in Scheme 1, Probe **1** was simply obtained by the reaction of 9,10-bis[(E)-2-(pyridin-4-yl)vinyl]anthracene with 1-bromoctane in DMF solution in high yield (80%). By slow evaporation of the solvent from a solution of probe **1** in CH_3CN, brown-red single crystals were successfully obtained, which were subjected to X-ray diffraction analysis. In the solid state, we first found that the molecular conformations of the anthracene core of the probe **1** maintained a large torsion angle up to 53.1° (Fig.1a and Fig.S1). At first glance, the molecules are seen to adopt a stacking mode similar to J-type aggregation and H-type aggregation along the *a* and *b* axis (Fig. S2-S3) in each column, respectively. However, the distances (d1, d2, d3) between the anthracene cores of the two adjacent molecules in the different aggregation modes were measured as 4.32 Å, 6.41 Å and 8.04 Å, respectively, and there was almost no overlap between the central anthracene planes, indicating an absence of π–π interactions in the crystals of **1**. Closer inspection suggested that the counter anion Br^- plays an important role in the supramolecular aggregation. For example, the main interactions between adjacent molecules in crystals of probe **1** were found to be C-H···Br^- interactions. In a single molecular column, C-H···Br^- interactions are formed between two molecules with



probe 1

Scheme 1 Chemical structure of probe **1**.

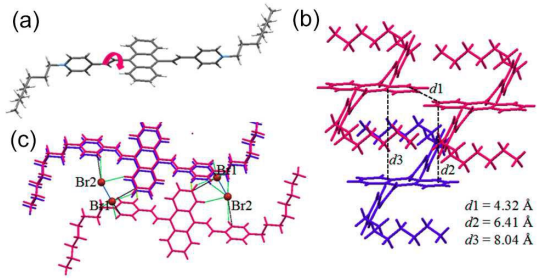


Fig. 1 (a) The configuration of one molecule of **1** in the crystals. (b) Stacking modes of the anthracene planes in the adjacent molecules of **1**. (c) Noncovalent interactions between molecules of **1** in the crystal.

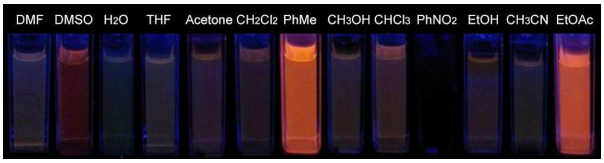


Fig. 2 Photographs of solution of **1** (0.1 mM) in different solvents under UV light at 365 nm.

interaction distance in the range 2.573~2.993 Å, whereby the Br^- acts as the H acceptor and the corresponding anthracenyl core and the pyridinium moiety of an adjacent molecule acts as H donors. In addition, some weak anion–π interactions with distance in the range 3.191~3.514 Å are also formed by Br^- anions and the aromatic moieties on the probe **1**. Furthermore, it is worth noting that although there are almost no π–π interactions in probe **1** in the solid state, the crystals exhibited quite strong fluorescence emission at around 626 nm (Fig. S4). The inhibition of vibrational relaxation in the solid aggregation state is assumed to be the origin of the high fluorescence emission in the crystalline molecular system.

To gain detailed insight into the AIE behaviour of probe **1** in solution, its fluorescence spectral properties were systematically evaluated in various solvents. Interestingly, possibly due to the solubility of the positively charged gemini-like **1** in both organic and aqueous media, no solvent-dependent AIE was observed when we carried out such experiments at a low concentration of **1** of 1×10^{-5} M. However, probe **1** exhibited different AIE fluorescence properties in different solvents at a higher concentration of 1×10^{-4} M. As shown in Fig. 2, in PhMe and EtOAc, it showed strong yellow fluorescence at around 592 nm and 605 nm, respectively. In DMSO, it exhibited very weak red fluorescence at around 625 nm and no turn-on fluorescence was observed in other solvents (Fig. S5). This observation indicated the operation of a kind of novel AIE effect in probe **1** in solution.

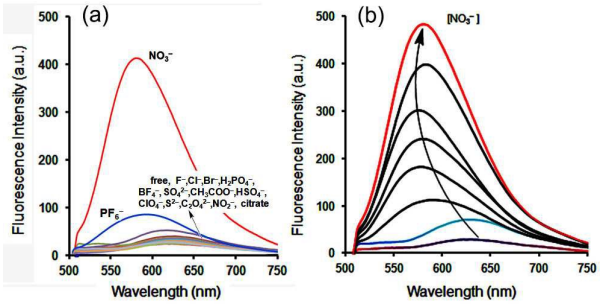


Fig. 3 (a) Response emission intensities of **1** (10 μM) for various sodium salts (0.1 mM) in 10 mM HEPES buffer (pH 7.4) at 298 K. (b) Fluorescence emission changes of 1 (10 μM) with various concentrations of $NaNO_3$ (0–0.1 mM) in 10 mM HEPES buffer (pH 7.4) at 298 K.

Fig. 4 Optical changes in fluorescence emission of **1** (10 μM) with various sodium salts (0.1 mM) in 10 mM HEPES buffer (pH 7.4) at 298 K under UV light at 365 nm. Left to right: F^-, Cl^-, Br^-, $H_2PO_4^-$, BF_4^-, SO_4^{2-}, NO_3^-, CH_3COO^-, HSO_4^-, ClO_4^-, S^{2-}, NO_2^-, PF_6^-, $C_2O_4^{2-}$, and citrate (sodium salts).

As mentioned previously, our recent study indicated that the phenylenevinylene pyridinium cation motif bearing the same alkyl chain shows excellent fluorescent sensing of NO_3^- anion with the assistance of protons in water.[14] In the present work, an anthracene group has been appended to the structure to give **1** in order to introduce a twisted intramolecular charge transfer (TICT) property, which was supposed to beneficial for the target analytes. An anion recognition experiment was therefore conducted by measuring the fluorescence variation of probe **1** (10 μM) in neutral aqueous solution upon the addition of 10 equiv. of F^-, Cl^-, Br^-, NO_3^-, $H_2PO_4^-$, SO_4^{2-}, HSO_4^-, BF_4^-, CH_3COO^-, ClO_4^-, S^{2-}, NO_2^-, PF_6^-, $C_2O_4^{2-}$, and citrate anions as their sodium salts. As can be seen from Fig. 3a, NO_3^- turned on the fluorescence and induced an about 25-fold enhancement as measured at 581 nm, whereas the other anions caused little or no changes. Fluorescence titration of **1** in neutral aqueous solution with different concentrations of NO_3^- (Fig. 3b) gave a linear relationship between the fluorescence of **1** at 581 nm versus the amount of anions added. The detection limit of **1** for NO_3^- was calculated to be 4.75×10^{-7} M (Fig. S6). Notably, no significant interference in the detection of NO_3^- with probe **1** was observed in the presence of most other common competitive anions, amino acids or metal ions in the real test environment (Fig. S7-8), indicating that probe **1** shows a highly selective fluorescent response to NO_3^- that can be clearly discerned by the naked eye under UV light (Fig. 4).

Meanwhile, it should be noted that a similar fluorescence response of **1** to NO_3^- over other tested anions was also observed in further experiments conducted in acidic (pH 3.0) or basic (pH 10.0) aqueous solutions (Fig. S9). This result further implied that probe **1** could be used as a selective chemosensor to monitor NO_3^- in the aqueous environment

Table 1. Thermodynamic parameters for the ionic interaction-triggered AIE of probe **1** with NO_3^- in different pH values.

experiment	ΔG^o/(kJ·mol^{-1})	ΔH^o/(kJ·mol^{-1})	$T\Delta S^o$/(kJ·mol^{-1})
pH 7.4	-26.20±0.07	-8.28±0.03	17.92±0.04
pH 3.0	-27.00±0.06	-2.56±0.03	24.44±0.03

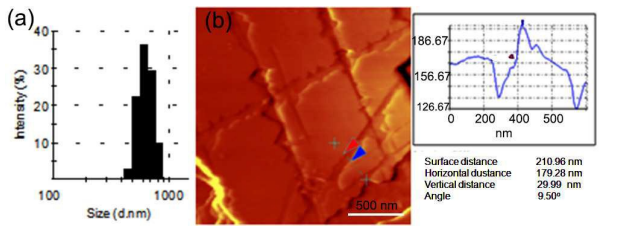


Fig. 5 (a) DLS data and (b) AFM image of the aggregation of **1**(10 μM) with NO_3^- (0.1 mM) in aqueous solution (pH 7.4) at 298 K.

over a wide pH range. To date, many excellent fluorescent probes with near-neutral response behaviour[5] have been exploited for applications in environmental and biological systems. Unfortunately, little research has been reported on the development of probes with tolerance to cover the whole acidic, neutral and basic region. As a matter of fact, in Nature, the organelles of cells have various pH values. For example, it has been reported that mitophagy, the specific autophagic elimination of mitochondria, involves a marked change in mitochondrial pH (approximately pH 4–8), which is directly associated with the pathogenic state.[15] Accordingly, the development of new chemosensors that allow continuous visualization of the physiological and pathological processes of biological species at various intracellular pH values is necessary and important.[16] The ionic interaction-triggered NO_3^- recognition described here provides an excellent complement to the current anion-sensing chemistry.

The sensing mechanism of **1** for NO_3^- anion in aqueous solution can be ascribed to ionic interaction-triggered aggregation, which results from the subtle interplay between the electrostatic ionic bonding of the nitrate anion with the positively charged pyridinium moiety, the hydrophobic effect of the aliphatic chains and the π-stacking of the divinylanthracene-derived fluorophore. The fluorescence turn-on emission around 581 nm can be attributed to the overlap between the central anthracene planes during the molecular aggregation. In order to better delineate the roles of the abovementioned noncovalent interactions in the ionic interaction- induced AIE assemblies, isothermal titration calorimetry (ITC) experiments (Fig. S10) were conducted at room temperature in acidic and neutral aqueous solution respectively, to quantify the enthalpic and entropic contributions to the binding interactions of the probe **1** and the NO_3^- anions. As shown in Table 1, the results ($|-T\Delta S^o| > |\Delta H^o|$) indicated that the interaction of **1** with NO_3^- was driven mostly by entropy and to some extent by enthalpy under both pH conditions.[17] The high entropy gain demonstrated a strong

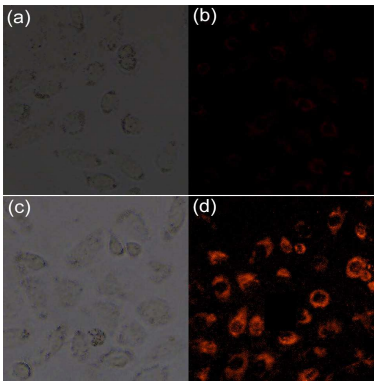


Fig. 6 (a) Bright-field image and (b) fluorescence images of the HeLa cells in the presence of 1 (10 μM) in PBS buffer at 37 °C for 30 min; (c) Bright-field image and (d) fluorescence images after incubation with $NaNO_3$ (10 equiv.) for another 20 min.

hydrophobic effect in the aggregation process of **1** with NO_3^-. Interestingly, it can be further noted that the contribution from enthalpy ($|\Delta H^o|$) of probe **1** with NO_3^- increased on going from acidic to neutral conditions, implying strong electrostatic interaction under neutral solution. On the contrary, in acidic solution, the entropy term ($|-T\Delta S^o|$) was larger, suggesting that protons have the ability to assist the hydrophobic effect of the probe.[18] In other words; the ionic assembly is more easily triggered by the anions in acidic solution.

In an effort to gain more information on the aggregation behaviour of the probe, dynamic light-scattering (DLS) experiments were performed to study the size distributions of the ionic interaction induced recognition assembly in solution (Fig. 5a). An aqueous solution of **1** (10 μM) and $NaNO_3$ (100 μM) at pH 7.4 exhibited an average hydrodynamic diameters of 610 nm, indicating that aggregation-induced polymers were formed. Furthermore, atomic force microscopy (AFM) images also clearly showed the presence of a regular ribbon-like assembled structure throughout the sample with a length of several micrometers and a height of about 30 nm (Fig. 5b). Therefore, based on these observations, ionic interaction-induced ribbon-like aggregation of **1** with NO_3^- was evidently confirmed.

To demonstrate the potential application of probe **1** for the detection of NO_3^- in biological media, fluorescence microscopy studies were carried out using HeLa cells. When HeLa cells were incubated with **1** (10 μM) in PBS buffer for 30 min at 37 °C, only weak emission was exhibited in the cells (Fig. 6b). After the cells were subsequently incubated with NO_3^- (10 equiv.) at 37 °C for a further 20 min, a strong yellow fluorescence was clearly seen (Fig. 6d). The bright-field images (Fig. 6a and 6c) showed that the cells were alive during the incubation period. These results indicated that probe **1** could penetrate the cell wall and be used for intracellular NO_3^- imaging in vitro.

Conclusions

In conclusion, we have studied the synthesis and properties of a new AIE-based turn-on fluorescent probe **1** for NO_3^- with a divinylanthracene group as the crucial sensing fluorophor. The probe **1** exhibits high selectivity for NO_3^- over various anions and bio-relevant analytes in aqueous solution over a wide pH range, which is attributed to the strong affinity of NO_3^- towards the positively charged pyridinium moieties through ionic interaction with the cooperation of the hydrophobic effect of probe **1**. Importantly, the gemini surfactant-like probe has been successfully applied to the turn-on fluorescence imaging of NO_3^- in living cells. Our work demonstrates that the ionic interaction-triggered AIE could be an efficient strategy to address the challenge of anion recognition in aqueous solution and biological systems over a wide pH range.

Acknowledgements

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Graphical Abstract and Text

A new type of AIE-based turn-on fluorescent probe **1** was reported to highly selective detection of NO_3^- anion in aqueous solution and living cells by virtue of ionic interaction.