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“Off-on” aggregation-based fluorescent sensor for the detection of chloride in water†

Michelle M. Watt, Jeffrey M. Engle, Kurtis C. Fairley, Timothy E. Robitshek, Michael M. Haley*, and Darren W. Johnson*

Department of Chemistry & Biochemistry and Materials Science Institute, University of Oregon, Eugene, OR 97403-1253, USA

Abstract

Receptors selective for anions in aqueous media are a crucial component in the detection of anions for biological and environmental applications. Recent sensor designs have taken advantage of systems known to aggregate in solution, eliciting a fluorescent response. Herein, we demonstrate a chloride-selective fluorescent response of receptor 1^+ , based on our well-established class of 2,6-bis(2-anilinoethynyl)pyridine bisureas. The fluorescence intensity ratio of $1^+ \cdot \text{Cl}^-$ aggregates in water is four times larger than the next most fluorescent anion complex, $1^+ \cdot \text{ClO}_4^-$. In addition, ^1H NMR spectroscopic titrations demonstrate 1^+ binds chloride more strongly than other biologically relevant anions in solutions of both $\text{DMSO}-d_6$ and 50/50 $\text{DMSO}-d_6/\text{MeCN}-d_3$.

Introduction

Supramolecular sensors for the detection of anions have received considerable attention over the past two decades.^{1,2} Such systems typically exploit non-covalent interactions between a guest molecule and a host molecule to induce a change in the host (*e.g.*, shift of NMR resonance, color change, fluorescence response, electrochemical behavior, *etc.*). Supramolecular sensors are advantageous when compared to chemodosimeters because non-covalent interactions are reversible, providing dynamic monitoring of an analyte. Arguably the most powerful of these sensors exploit a fluorescence change due to their inherent sensitivity.³

Existing fluorescent organic sensors for halides are either based upon a 6-methoxyquinolinium scaffold^{4–6} or modified yellow fluorescent protein.^{7,8} Both classes of sensors undergo collisional quenching in the presence of a halide, allowing the concentration of analyte to be ascertained using the Stern–Volmer relationship.⁹ While these sensors are widely used and commercially available, turn-off sensors suffer from high background emission; therefore, it is desirable to develop a turn-on sensor to allow for improved spatial resolution of the target ion. Furthermore, existing sensors are susceptible to interference from other halides, “pseudohalides”, and dissolved oxygen, thus making more selective alternatives attractive.^{4,9}

†Electronic supplementary information (ESI) available: Synthesis and spectroscopic data of **3**, **1**, 1^+ , titration data for 1^+ .

dwj@uoregon.edu, haley@uoregon.edu; Fax: +541-346-0487; Tel: +541-346-1695.

“Aggregation induced emission” (AIE) dyes or dye aggregates with active photophysical properties typically contain highly conjugated backbones with moieties capable of having their inherent rotational freedom restricted by non-covalent interactions or crystal packing forces.^{10,11} Often acting through J-aggregation, these systems have been known since at least 1935¹¹ and have recently found use as fluorophores for sensing analytes.^{11,12} Under most conditions the molecules rotate freely and are non-fluorescent, but upon application of an external or internal stimulus, such as a change in solvent or temperature, rotational freedom becomes restricted.¹⁰ Ultimately the molecules aggregate and an atypical turn-on fluorescent response occurs. AIE has been used as a sensing platform for analytes such as cyanide,¹³ carbohydrates,¹⁴ amino acids,¹⁵ CO₂,¹⁶ and electron deficient aromatics.¹⁷ Aggregation-based fluorescence responses could allow for turn-on fluorescence sensors to be further developed for use in detecting analytes, such as halides, typically viewed as quenching agents.¹⁰

Our previously reported bis(arylethynyl)pyridine-based compounds have shown considerable promise towards selective turn-on anion sensing.^{18–21} The synthesis and characterization of a turn-on sensor (**1**⁺), which is activated by aggregation with anions and selective for chloride over other biologically relevant anions in water under acidic conditions, is reported herein. This is a rare example of a fully organic, turn-on fluorescent sensor selective for chloride in water. Chloride’s importance is increasingly appreciated as more studies demonstrate its involvement in biological processes, including regulation of cellular volume and nerve transduction.^{22–24}

Results and discussion

Synthesis of receptor **1** is achieved through a typical Sonoga-shira cross-coupling and introduction of a water-solubilizing group in the final step (Scheme 1). Reaction of two equivalents of urea **2**²⁵ with 2,6-dibromo-4-nitropyridine in the presence of Pd(0) and CuI provides precursor **3** in 84% yield. S_NAr reaction of **3** with 2-dimethylaminoethanol and potassium carbonate produces **1** in 67% yield. Previous results with similar pyridine receptors^{18–20} demonstrated a positive fluorescent response to chloride and other anions upon the protonation of the host. The addition of more than two equivalents of trifluoroacetic acid (TFA) in acetonitrile provides the final water-soluble chloride sensor, denoted here as “**1**⁺” (the plus sign symbolizes only the protonated state of the receptor, not the charge of the receptor complex).[‡] Unprotonated receptor **1** is not soluble in aqueous solutions; however, pre-protonated **1**⁺ is soluble in water with 1% TFA up to ~0.5 mM; solubility is diminished to ~0.1 mM without the presence of 1% TFA.

Fluorescent response to anions

To determine selectivity of **1**⁺, excess equivalents (>500) of various anions§ as their sodium salts were added to 0.5 mM aqueous solutions of **1**⁺ in the presence of 1% TFA (Fig. 1).

[‡]**1**⁺ degrades as a solid and therefore cannot be stored in its protonated form. We have verified the stability of **1**⁺ in DMSO for over 6 months, but have not yet confirmed the stability of **1**⁺ in water for extended periods of time. §Dihydrogen phosphate is excluded from these results due to its likely fully protonated state at the pH of the solutions containing excess TFA, as well as experimental NMR evidence indicating its ability to deprotonate the receptor when excess TFA is not present.

Upon addition of a sodium salt to 1^+ , a visible color change from pale to dark yellow is observed, depending on the concentration or equivalents of chloride. A notable distinguishing feature is the presence of aggregates in certain samples, which coincides with fluorescence turn-on. The presence of small spherical aggregates was confirmed by SEM (ESI). Excitation at 365 nm *via* a hand-held long-wave UV lamp illustrates the selective response of the protonated host to chloride, resulting in a change from a nearly imperceptible blue to a bright yellow-green fluorescence (Fig. 1c). Most of the other anions investigated produced little to no turn-on response, except for sodium fluoride and sodium perchlorate—significant anions with regards to their environmental impact but not typically a biologically relevant interferent with regards to sensing chloride in a cellular environment. This selectivity is further highlighted in the emission spectra collected when the complexes are excited at $\lambda_{\text{max}}(1^+ \cdot \text{Cl}^-) = 425 \text{ nm}$ (Fig. 1a,b); the intensity ratio of $1^+ \cdot \text{Cl}^-$ is four times larger than the next most fluorescent anion complex, $1^+ \cdot \text{ClO}_4^-$. In addition, $1^+ \cdot \text{Cl}^-$ is red-shifted by 75 nm in comparison to 6-methoxyquinolinium derivatives currently commercially used, making it more amenable for use in cells.

Fluorescent character of $1^+ \cdot \text{Cl}^-$ aggregates

To demonstrate the emission and aggregation of 1^+ in the presence of chloride, the percent volume of water was varied with a solvent capable of solubilizing $1^+ \cdot \text{Cl}^-$. Aggregation was observed when mixing 1^+ with a source of chloride in nearly all solvents investigated except for DMSO. Due to the high solubility of the receptor in DMSO, the AIE character was determined at relatively high concentrations of 1^+ and equivalents of chloride. A series of 0.5 mM 1^+ and 1.5 M LiCl solutions were made with the volume ratio of water ranging from 0% to 90% in 1% TFA–DMSO. The concentration of TFA and 1^+ were held constant throughout the solutions and the equivalents of chloride are nearly identical. A change in the yellow intensity of the solutions under visible light is, once again, observable upon mixture with chloride (ESI). Receptor 1^+ possesses a blue fluorescence in DMSO but switches closer to a white fluorescence upon the addition of chloride (ESI). As water is introduced to the DMSO solution, up to 70%, this fluorescence is quenched (Fig. 2). At 80% water in DMSO, $1^+ \cdot \text{Cl}^-$ begins to aggregate and fluorescence is dramatically turned on. Once 90% water is reached, the solution is still highly fluorescent and the visible concentration of aggregates is further increased (Fig. 2b); however, the measured fluorescence at an excitation of 425 nm is quenched in comparison to the 80% water solution (Fig. 2a). This observation could be caused by the large concentration of aggregates (which typically results in quenching for non-AIE active compounds).

^1H NMR spectroscopic titrations of 1^+

While aggregation in this class of receptors is beneficial for a fluorescent turn-on response to anions such as chloride, it is often detrimental in the determination of association constants, making it difficult to compare the strength of binding to current receptors. The overall weak association in strong hydrogen bonding solvents and the time-dependent nature of the fluorescent response of $1^+ \cdot \text{Cl}^-$ lead to the investigation of binding *via* ^1H NMR spectroscopy in d_6 -DMSO.

Initial titration experiments were performed at 1.5 mM of $\mathbf{1}^+$ in d_6 -DMSO spiked with 0.5% H_2O . Addition of TBA salts of nitrate, iodide, hydrogen sulfate, and perchlorate resulted in little to no change in chemical shift of any protons (ESI). Dihydrogen phosphate, as mentioned previously, deprotonates the host, indicated by the immediate loss of the $\text{R}_3\text{N}^+\text{H}$ resonance upon the first addition of salt and the change of chemical shifts to match $\mathbf{1}$ at the end of the titration (ESI). Titration of TBA chloride and bromide result in the downfield shift of four proton resonances on $\mathbf{1}^+$: $\text{R}_3\text{N}^+\text{H}$, HN_{urea} , $\text{H}_2\text{N}_{\text{urea}}$, and HC_{Py} . These four shifts were globally fit to 1: 1 association using non-linear regression in MatLab.²⁶ Titration results in 0.5% $\text{H}_2\text{O}/d_6$ -DMSO, however, were inconsistent and correspond to possible additional equilibria[¶] and weak association overall ($K_a \sim 100 \text{ M}^{-1}$) in a highly competitive solvent system, which still demonstrates a selectivity for chloride over other biologically relevant anions investigated.

To ascertain association constants of $\mathbf{1}^+$ more easily, less competitive solvents were used in conjunction with d_6 -DMSO. A 1.5 mM solution of $\mathbf{1}^+$ in 50% $\text{CD}_3\text{CN}/d_6$ -DMSO produced a clean 1: 1 binding isotherm for the addition of TBACl (Fig. 3) and TBABr (ESI) determined from ^1H NMR spectroscopic titrations. An induced upfield shift and corresponding sharpening of the residual water resonance indicates the displacement of water from the binding pocket upon the addition of chloride or bromide. The presence of additional peaks during the titration was not observed within this solvent system and the shifts of $\text{R}_3\text{N}^+\text{H}$, HN_{urea} , $\text{H}_2\text{N}_{\text{urea}}$, and HC_{Py} were globally fit to 1: 1 association using non-linear regression in MatLab.²⁶

Similar to observations of titrations in 0.5% $\text{H}_2\text{O}/d_6$ -DMSO, the protonated ethanolamine ($\text{R}_3\text{N}^+\text{H}$) moiety produces the largest change in chemical shift observed for the addition of chloride ($\delta \approx 1.61 \text{ ppm}$) while the pyridine resonance (HC_{Py}) produces the second largest shift ($\delta \approx 0.38 \text{ ppm}$, Fig. 3). The globally fit association constant for chloride was determined to be $300 \pm 10 \text{ M}^{-1}$. Bromide also fit to a 1: 1 equilibrium with the largest changes in chemical shifts also corresponding to $\text{R}_3\text{N}^+\text{H}$ and HC_{Py} ($\delta \approx 0.45$ and 0.34 ppm , respectively), but exhibits weaker binding than chloride with an association constant of $40 \pm 1 \text{ M}^{-1}$. In addition to confirming the selectivity for chloride in highly competitive solvent systems, NMR titrations are able to provide insight into the multiple binding conformations and possible routes to aggregate formation.

Binding conformations of $\mathbf{1}^+$

Most of our bisurea arylethynyl systems, particularly the pyridine- and phenyl-based receptors, typically bind in a U conformation,^{20,27,28} leading us to hypothesize receptor $\mathbf{1}^+$ would adopt a similar U conformation with a five-point binding site involving the electrostatic pyridinium moiety (Fig. 4). However, crystal and solution studies have also demonstrated the existence of alternative W and S conformations in this general class of bisurea arylethynyl receptors.^{29–31}

[¶]The titration of the seemingly 1: 1 chloride association ends at ~15 equivalents. Beyond 15 equivalents new peaks appear in the ^1H NMR spectra indicating the presence of an additional equilibrium, slow on the NMR time scale. In the case of bromide, however, a new peak appears before the titration is finished at ~30 equivalents of anion.

Binding conformation would be affected by the protonation state of $\mathbf{1}^+$ as two sites are available for protonation: the pyridine and the *N,N*-dimethylamino moiety. While data only provide confirmation of the protonated amine (^1H NMR spectroscopy), the observed increase in yellow color intensity upon addition of excess TFA in solution and when $\mathbf{1}^+$ is isolated as a solid suggests the presence of a pyridinium based on our previous receptors of this class.^{20,21} Overall, two conformations can be envisioned for 1: 1 binding: **U** and **S/W** (the second arm would freely rotate in solution essentially providing either an **S** or **W** type arrangement).

Based on the observed changes in ^1H chemical shifts for both bromide and chloride, we can speculate further upon the binding conformations of $\mathbf{1}^+$ with chloride or bromide (**U**, **S**, or **W**, Fig. 4) in DMSO solution. Considering the largest proton resonance shifts, and therefore typically the strongest hydrogen bonds, occur with $\text{R}_3\text{N}^+\text{H}$ and HC_{Py} , conformation **1:1 U** is highly unlikely (Fig. 4). Chloride must sit inside the cleft formed from an **S** or **W** arrangement in order to H-bond to the $\text{R}_3\text{N}^+\text{H}$ and HC_{Py} protons, similar to what is observed when our related bipyridine-based receptor binds chloride or bromide (referred to as the **Z** arrangement).³⁰ As discussed, additional equilibria arise during the titration of bromide or chloride into $\mathbf{1}^+$, likely indicating a competing 1: 2 interaction. What is unclear is whether this secondary equilibrium occurs with the pyridinium NH proton (**1:2 S**) or CH proton (**1:2 W**). While the binding conformation is of interest, the important aspect of this system lies within its strong and selective fluorescent response to chloride.

Conclusions

The quantitative detection of chloride using a small molecule probe with a positive fluorescence response is a challenging target for molecular probe development. Fully organic sensors for aqueous chloride typically undergo fluorescence quenching or suffer from competition with other anions. By using chloride as a template for fluorophore aggregation, receptor $\mathbf{1}^+$ offers a route to overcome these obstacles. Other biologically relevant anions investigated appear to template non-emissive or significantly blue shifted aggregates in water, when produced. Titrations in a 1: 1 solution of d_6 -DMSO/ d_3 -MeCN further demonstrated the selectivity of $\mathbf{1}^+$ for chloride in competitive media. While this receptor still suffers from inconsistencies likely dependent on pH or analyte/sensor concentration, $\mathbf{1}^+$ provides proof of concept towards the further design of aggregation-based, small molecule turn-on chloride cellular sensors in water. If aggregation size or growth can be controlled, this method is capable of providing a linear response to chloride concentration to realize this goal, and as such, $\mathbf{1}^+$ is influential for our future efforts in this area.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Emission profiles of host 1^+ with various anions depicted through (a) intensity ratios in comparison to 1^+ without the presence of anion, (b) fluorescence spectra, and (c) visual emission under a long-wave, 365 nm fluorescent lamp.

Fig. 2.

Emission profiles of $1^+\cdot\text{Cl}^-$ in solutions of 1% TFA in H₂O/DMSO mixtures with water ranging from 0–90% depicted through (a) the fluorescence spectra excited at 425 nm, (b) a plot of the total intensity from 440–740 nm, and (c) the visual emission under a long-wave, 365 nm fluorescent lamp.

Fig. 3. Stacked ^1H NMR spectra from the titration of TBACl into a 1.5 mM solution of $\mathbf{1}^+$ in 50/50 $\text{CD}_3\text{CN}/\text{d}_6\text{-DMSO}$.

Fig. 4.
Possible binding conformations for 1 : 1 or 1 : 2 arrangements of $1^+\cdot X^-$.

Scheme 1.
Synthesis of receptor 1.