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Anion Recognition

Anion Detection by a Fluorescent Poly(squaramide): Self-Assembly of Anion-Binding Sites by Polymer Aggregation**

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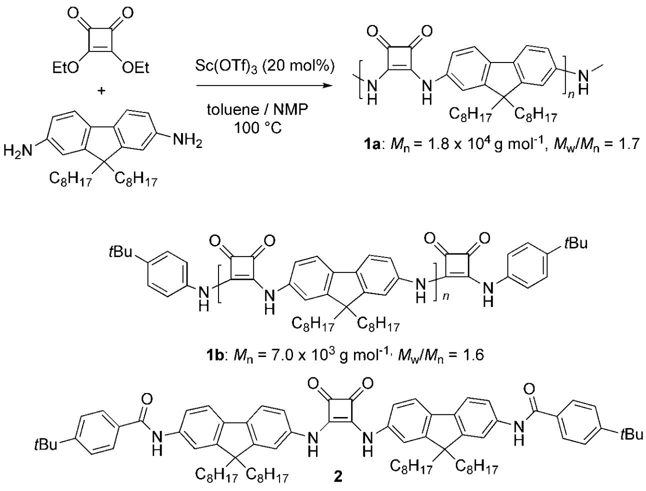
The selective recognition and sensing of anions has been the subject of intensive research effort, motivated by applications in medical diagnostics, environmental and industrial monitoring, and nuclear waste cleanup.[1] Selectivity and affinity may be achieved by preorganization of binding groups in an orientation complementary to the geometry and charge distribution of the anionic guest: sulfate- and phosphate-binding proteins represent impressive illustrations of this principle.[2] While there has been some progress toward synthetic hosts capable of high-affinity, selective anion recognition in competitive (aqueous) environment,[3] the synthesis of preorganized targets having the ideal number and orientation of binding groups for a given analyte is often difficult,[4] and rates of guest binding and release may become problematic. An attractive solution to this problem employs reversible processes (noncovalent or dynamic covalent interactions) as the basis for receptor self-assembly. Self-assembled capsules,[5] dynamic covalent libraries,[6] and coordination complexes[7–9] capable of anion binding are successful implementations of this concept.

Here, we describe experiments demonstrating that aggregation of an organic polymer composed of repeating hydrogen-bond donor groups may be exploited to achieve remarkable enhancements in anion affinity and selectivity. In particular, a polymer based on the 3,4-diaminocyclobutene-1,2-dione (squaramide) functional group shows a selective "turn-on" fluorescence response to dihydrogenphosphate ($H_2PO_4^-$) ions in the competitive medium 10% water in *N*-methylpyrrolidinone (NMP). The dual role of the squaramide groups in controlling both the aggregation of the polymer and its anion-responsive properties results in complex behavior, including cooperativity in analyte binding. Comparison with a non-polymeric reference compound indicates that incorporation of the squaramide group into a polymer results in an unprecedented alteration of anion selectivity as well as an enhancement in anion sensitivity.

Our investigations began with the synthesis of poly(squaramide) **1a** (Scheme 1). This material is unlikely to be capable of long-range exciton transport, a property that has been exploited to generate signal "gain" in conjugated polymer-based detection schemes.[10,11] However, we sought to test the hypothesis that **1a** could display anion-responsive properties resulting from the precedented aggregation behavior of polyamides in general,[12] and of materials containing squaramide[13] and urea[14] groups in particular, coupled with the known anion-binding properties of these functional groups.[15] Squaramides bind tightly to anions even in competitive media, a property attributed to the high acidity of squaramide NH groups relative to those of amides or (thio)ureas.[16]



Scheme 1. Preparation of poly(squaramides) by Lewis acid catalyzed condensation.

Polymer **1a** was prepared by scandium(III) triflate-catalyzed condensation of 9,9-dioctylfluorene-2,7-diamine and diethyl squarate employing a modification of our recently developed procedure for the preparation of aniline-based squaramides.[16b] Under Lewis acid catalyzed conditions, the poly(squaramide) was obtained with good molecular weight and polydispersity ($M_n = 1.8 \times 10^4$ g mol^{-1}, $M_w/M_n = 1.7$) as judged by gel permeation chromatography (GPC) in 0.2 wt% LiCl/NMP with poly(methyl methacrylate) standards. Polymerization in the presence of 4-*tert*-butylaniline yielded polymer **1b** carrying defined end groups. Values of M_n for **1b** determined by ^{1}H NMR spectroscopy (5.0×10^3 g mol^{-1}) and GPC (7.0×10^3 g mol^{-1}) were in good agreement. These

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materials, which are soluble in amide solvents such as NMP and *N*,*N*-dimethylformamide (DMF), represent the first examples of soluble, well-defined squaramide-based polymers, a new class of poly(amides).[17] The unusual anion sensory properties of these materials are discussed in the following paragraphs; experiments probing their mechanical and electronic properties are underway.

The interactions of polymer **1a** with anionic analytes were studied by fluorescence spectroscopy in 10% water/NMP, a competitive medium in which anion recognition by neutral hosts represents a challenge. Addition of tetra-*n*-butylammonium dihydrogenphosphate ($Bu_4N^+H_2PO_4^-$) to solutions of **1a** resulted in an increase in emission intensity (Figure 1a). This effect was evident to the naked eye upon irradiation of solutions of **1a** in the presence or absence of $H_2PO_4^-$ ions with a hand-held UV lamp (Figure 1a, inset).

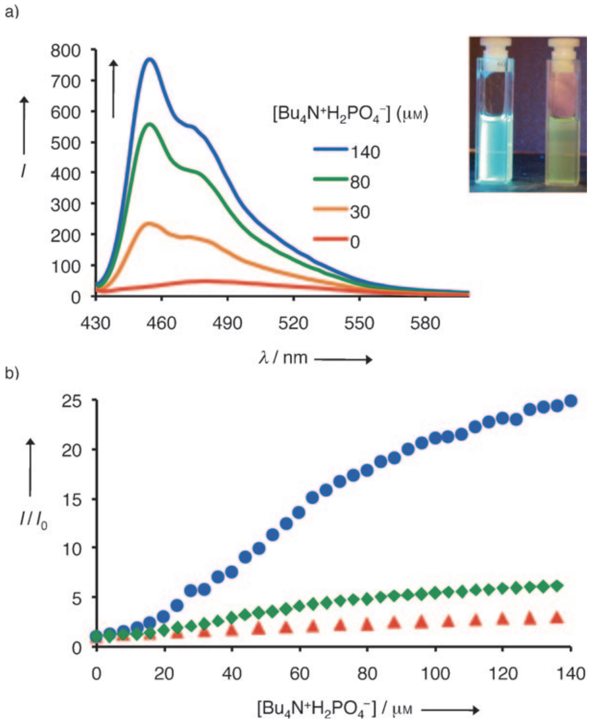

Figure 1. a) Fluorescence response of polymer **1a** to $Bu_4N^+H_2PO_4^-$ (10% water in NMP solvent; $\lambda_{ex} = 415$ nm); b) Concentration dependence of the fluorescence response ($\lambda_{em} = 453$ nm) of **1a** (blue ●) **1b** (green ◆), and **2** (red ▲) to $Bu_4N^+H_2PO_4^-$.

A graph of fluorescence intensity as a function of $H_2PO_4^-$ concentration shows the sigmoidal shape characteristic of a cooperative anion response, in which polymer–phosphate interactions trigger additional binding events (Figure 1b). A Hill plot indicates positive cooperativity for the **1a**–$H_2PO_4^-$ interaction ($n_H = 2.5$),[18] although the complex nature of the polymer–anion interaction (see below) likely precludes a straightforward application of this type of analysis.[19] While cooperativity is a hallmark of binding and recognition processes in biological systems,[20] its exploitation in synthetic receptors represents an emerging area of research.[21]

To evaluate the importance of the polymeric architecture of squaramide **1a**, compound **2** was prepared as a control receptor. Like polymer **1a**, squaramide **2** underwent an increase in fluorescence quantum yield upon addition of $Bu_4N^+H_2PO_4^-$ in 10% H_2O/NMP, but the cooperative response characteristic of **1a** was not observed. Instead, **2** showed behavior consistent with 1:1 binding ($K_a = 2.8 \times 10^3\,\mathrm{M}^{-1}$, determined by fitting changes in fluorescence intensity to a 1:1 binding curve).[18,22] Polymer **1a** is approximately four times more sensitive to phosphate ions than is **2**: the half-maximal fluorescence responses of **1a** and **2** were observed at $H_2PO_4^-$ concentrations of 50 mM and 200 mM, respectively. The magnitude of the fluorescence response of **1a** is also greater (maximum values of I/I_0 for **1a** and **2** were 25 and 5, respectively); this is largely a consequence of the lower fluorescence of **1a** as compared to **2** in the absence of analyte, likely due to aggregation of the former (see below). We attribute the enhanced emission of **1a** and **2** in the presence of $H_2PO_4^-$ ions to an intramolecular charge-transfer mechanism.

Incorporating the squaramide group into a poly(amide) backbone has a profound effect on the selectivity of system (Figure 2). The fluorescence response of polymer **1a** is selective for $H_2PO_4^-$ over other monovalent anions, including basic analytes such as fluoride and hydroxide. In contrast, model compound **2** shows the largest fluorescence response towards fluoride, followed by $H_2PO_4^-$ ions. The difficulty in developing receptors capable of the selective binding of $H_2PO_4^-$ ions in the presence of F^- has been documented previously.[23] Selective detection of inorganic phosphate ions in aqueous medium is of interest, both from the perspective of its relevance in biological systems and human health, and its role as a pollutant causing eutrophication of fresh water.[10d,24] It is noteworthy that the affinity and selectivity of polymer **1a**, which has no evident preorganized "binding site" for $H_2PO_4^-$ ions, compare favorably with those of state-of-the-art, uncharged receptors capable of phosphate binding in competitive medium.[25]

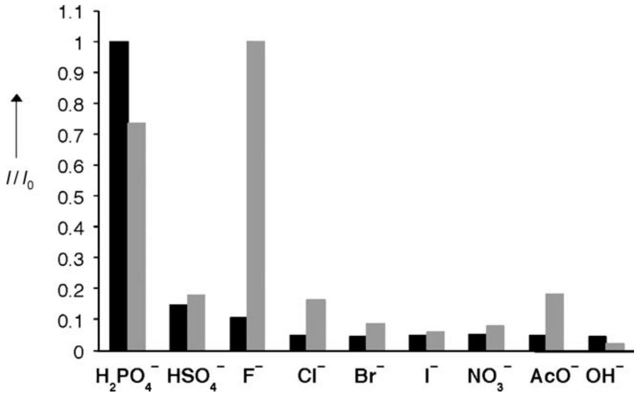

Figure 2. Normalized fluorescence response I/I_0 of polymer **1a** (black bars) and model compound **2** (gray bars) to anions X^- ($nBu_4N^+X^-$, 10% H_2O/NMP solvent; anion concentration 140 μM for **1a** and 1200 μM for **2**, respectively).

The unusual enhancements in affinity and selectivity observed upon incorporation of the squaramide group into polymer **1a** appear to be results of the interplay between polymer aggregation and analyte binding. As is typical of aromatic amide-based polymers, **1a** is prone to aggregation: GPC analysis with NMP as eluent resulted in an apparent M_n of $2.0\times10^6\,\mathrm{g\,mol^{-1}}$; upon addition of LiCl, which promotes the deaggregation of polyamides, the reported molecular weight of $1.8\times10^4\,\mathrm{g\,mol^{-1}}$ was determined. Temperature-dependent UV/Vis spectra in DMF and transmission electron microscopy (TEM) also indicated aggregation of **1a**.[18]

Dynamic light scattering (DLS) measurements in DMF solution demonstrated that addition of phosphate ions triggers the formation of well-defined aggregates (mean hydrodynamic radius 120 nm, PDI = 0.1; Figure 3a,b) from an initially heterogeneous distribution in its absence (low scattering intensity: hydrodynamic radius 50 nm, PDI = 0.3). The results of TEM experiments were in qualitative agreement with the solution-phase behavior: in the absence of $Bu_4N^+H_2PO_4^-$, aggregates of **1a** varying in size and shape were observed,[18] but images of **1a** in the presence of $Bu_4N^+H_2PO_4^-$ indicated spherical aggregates of 200–800 nm diameter (Figure 3c).[26] The larger of these aggregates were visible by laser confocal microscopy in DMF.[18] This behavior stands in contrast to previously reported deaggregation-based detection schemes employing conjugated polymers[10a,27] and urea-based oligomers.[14c]

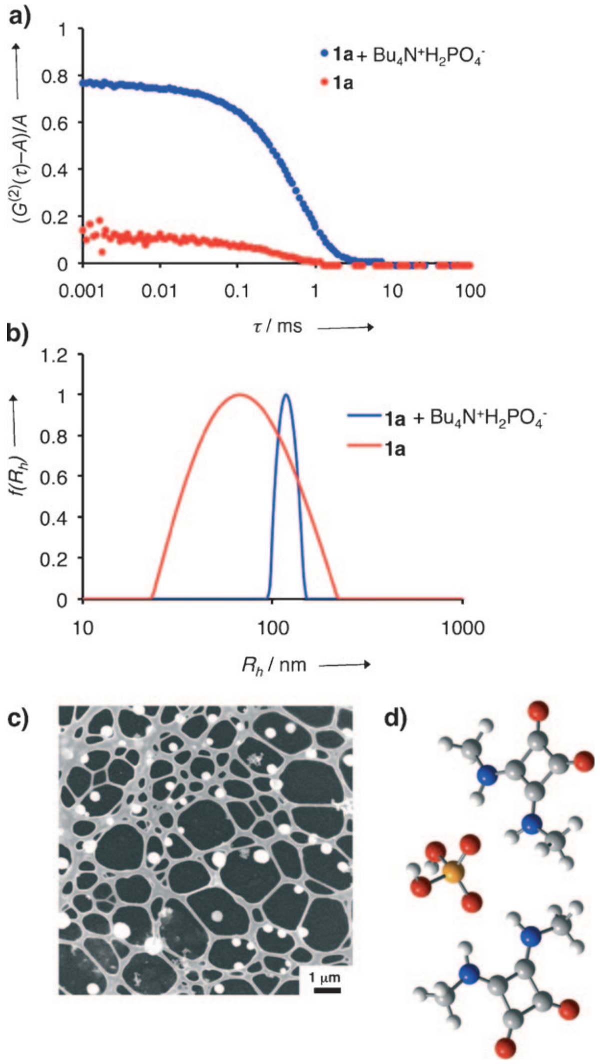

Figure 3. a) Autocorrelation functions and b) normalized CONTIN plots from DLS measurements at 90° on polymer **1a** (2.4×10^{-5} M) in the absence (red ●, ——) and the presence (blue ●, ——) of $Bu_4N^+H_2PO_4^-$ (2.4×10^{-3} M) in DMF. c) TEM image of a dried film of **1a** and $Bu_4N^+H_2PO_4^-$ (sample was cast from a DMF solution of **1a** (2.4×10^{-5} M) and $Bu_4N^+H_2PO_4^-$ (2.4×10^{-3} M)). Spherical polymer aggregates are clearly visible. The lacy pattern is the copper framework of the carbon-coated copper TEM grid. d) Calculated (B3LYP/6-311+G**) geometry of the 2:1 complex of *N*,*N'*-dimethylsquaramide and $H_2PO_4^-$.

It thus appears that anion-triggered reorganization of polymer aggregates gives rise to the cooperative response and altered analyte selectivity that characterize the poly(squaramide) system. We propose that $H_2PO_4^-$ ions are able to engage in hydrogen-bonding interactions with multiple squaramide groups, bringing polymer chains together in a manner that results in a cooperative anion response.[28] Consistent with this hypothesis, the magnitude and degree of cooperativity of the anion-induced fluorescence response increased as a function of polymer molecular weight (see Figure 1b and the Supporting Information). Precedent for this proposal includes the existence of urea-based receptors that bind to $H_2PO_4^-$ with a 2:1 urea:anion stoichiometry,[25a] and the observation of a squaramide host that undergoes sulfate-induced dimerization.[29] Calculations (B3LYP/6-311+G**) suggest a geometry for such a 2:1 squaramide:phosphate complex (Figure 3d). Recent studies suggest that secondary, attractive intra-receptor interactions are a powerful strategy for achieving high-affinity binding in competitive medium;[30] the present example appears to represent a new illustration of this principle.

The superior mechanical properties of poly(amides) that underlie their commercial utility are largely the result of interpolymer hydrogen-bonding interactions. This work demonstrates for the first time that polyamides may be employed for selective anion detection in competitive medium, and indicates that the aggregation behavior of this class of materials may offer significant benefits in the context of chemical sensing. Viewed in another light, this represents a system in which the supramolecular aggregation of a polymer is under the control of a specific small-molecule stimulus. Efforts to develop a more detailed mechanistic understanding of the anion-induced response, and to modify the polymer structure to achieve selective anion binding in still more competitive media, are ongoing in our laboratories.

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