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Interaction of Halide and Carboxylate Ions with 4,5-Diacetamidoacridine-9(10*H*)-one: Thermodynamics of Association and Deprotonation Events

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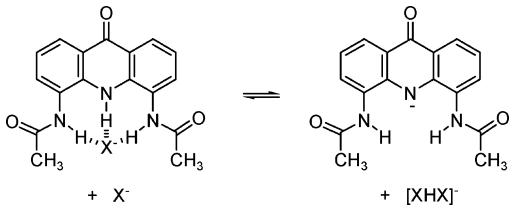
Received December 2, 2006



4,5-Diacetamidoacridine-9(10*H*)-one was prepared, and its interactions with halide and benzoate anions were studied using a combination of NMR, fluorescence, and isothermal titration calorimetry experiments. Whereas chloride and bromide exhibited simple association, both fluoride and benzoate exhibited initial entropy-driven association followed by an enthalpically favorable deprotonation of the receptor by a second equivalent of the anion.

Introduction

The development of anion receptors has become a field of substantial interest and activity.[1−3] Although some anion receptors have been based on metal complexes, the more common approach is based on multiple hydrogen bond donor groups to bind the target anion.[4] Receptors based on the latter approach generally function only in aprotic solvents where competing interactions with solvent are avoided. Large changes in absorbance and fluorescence spectra of some of these receptors upon anion addition has led to their investigation as possible anion sensors. It has recently been recognized that these large spectral changes are often due to deprotonation of the receptor/sensor by the basic anion rather than simple association.[5−10]

Receptors based on urea, thiourea, and guanidinium groups have been used quite successfully in the development of receptors for carboxylate, phosphate, and sulfonate ions.[11−14] These rely on a pair of hydrogen bonding interactions between the parallel N−H bonds of the receptor and a pair of oxygen atoms of the anion. Although similar receptors have also been used for binding of halide ions, the hydrogen bonds must be very nonlinear for interaction of both hydrogen bond donors with the single atom bearing the negative charge.[15,16] A variety

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10.1021/jo062467j CCC: $37.00
Published on Web 02/08/2007

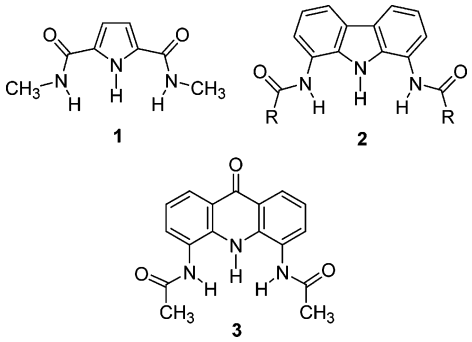

FIGURE 1. Structures of anion receptors.

of structures have been prepared to provide an array of hydrogen bond donor groups around a monatomic anion binding site including the 2,5-diamidopyrroles **1** and 1,8-diaminocarbazole amides **2** (Figure 1).[17−22] 4,5-Diacetamidoacridine-9(10*H*)-one **3** appears on the basis of visual inspection and computer modeling to have hydrogen-bonding groups arranged in an especially optimal convergent orientation for binding of a small monatomic anion. Compound **3** has been prepared for the purpose of characterization of the corresponding diamine, but its receptor properties have not been reported.[23] We report here studies of the interaction of **3** with halide and benzoate anions using a combination of NMR, fluorescence, and calorimetry experiments.

Results and Discussion

4,5-Diaminoacridone was prepared and converted to the bis-acetamide **3** following literature procedures.[23−25] Binding of **3** to halide ions as their tetrabutylammonium salts in DMSO was initially assessed by ^{1}H NMR. Upon titration with chloride ion, the signals assigned to the amide and acridone N-H protons experienced downfield shifts of 0.68 and 0.36 ppm, respectively, while the aromatic protons exhibited observable but smaller changes. Data analysis using the program EQNMR gave a fit consistent with 1:1 binding and gave an association constant of about 30 (±5) M^{-1}.[26] The changes in chemical shift induced by bromide were much smaller but also were fit to 1:1 binding and gave an association constant of about 3 M^{-1}. The accuracy of this value is limited as the curvature in the chemical shift versus concentration plot is small in the concentration range used, but the result is in good agreement with the value determined from fluorescence (see below). These association constants and the selectivity for chloride over bromide are similar to results reported for other receptors.[2] Introduction of fluoride ion caused severe broadening of the ^{1}H NMR signals such that precise changes in chemical shift and an association constant could not be evaluated. This may result from rapid proton exchange due to deprotonation in addition to simple association (see below).

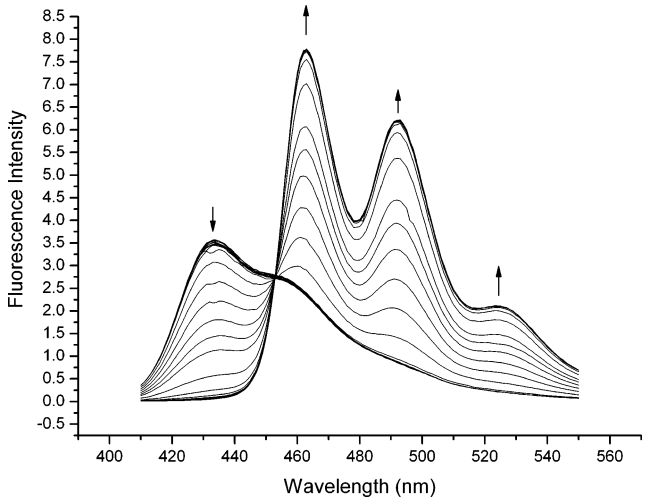

FIGURE 2. Fluorescence spectral changes of **3** with increasing fluoride ion concentration.

Fluorescence studies of the interaction of halide ions with **3** were conducted by observing emission spectra with excitation at 360 nm. Figure 2 shows the emission spectrum of **3** with increasing concentrations of fluoride ion. There was little change in the fluorescence spectrum upon addition of initial increments of fluoride. However, a dramatic change began to be observed as the ratio of fluoride ion to receptor reached 1 equiv, with the initial peak at 435 nm disappearing while new peaks appeared with maxima at about 465 and 495 nm, along with a much less intense peak near 530 nm. From these results it became apparent that although the expected noncovalent complex with fluoride ion was initially formed, upon further addition the fluoride ion acted as a base to deprotonate **3** with formation of the FHF^- ion, as recently proposed by Boiocchi et al. for urea-based receptors.[5] The sharp isosbestic point at about 455 nm indicates that the initial fluoride binding does not have a significant effect on the fluorescence of **3**. Fitting of the decrease in emission at 435 nm upon reaction of the second equivalent of fluoride with the initial fluoride complex gave an apparent equilibrium constant of about 10^6 M^{-1}, though there is some uncertainty in this value if the two steps are not fully distinct. The binding constant for the first equivalent of fluoride could not be directly evaluated from the fluorescence data because of the absence of an observable spectral change. However, since the initial binding event appears to go essentially to completion prior to the subsequent deprotonation step, the binding constant is substantially greater than 10^6 M^{-1}. This is consistent with reported binding constants for fluoride ion to urea-based receptors of 10^6 to $>10^7$ M^{-1}.[5−10]

Addition of chloride, as with fluoride, gave a concentration-dependent decrease in the emission peak at 435 nm but gave much smaller increases in emission at longer wavelength (Figure 3). This indicates that **3** is not being converted quantitatively to the anion under these conditions. The chloride ion concentration necessary to give a fluorescence change was much greater than the receptor concentration, and thus distinct reactions with

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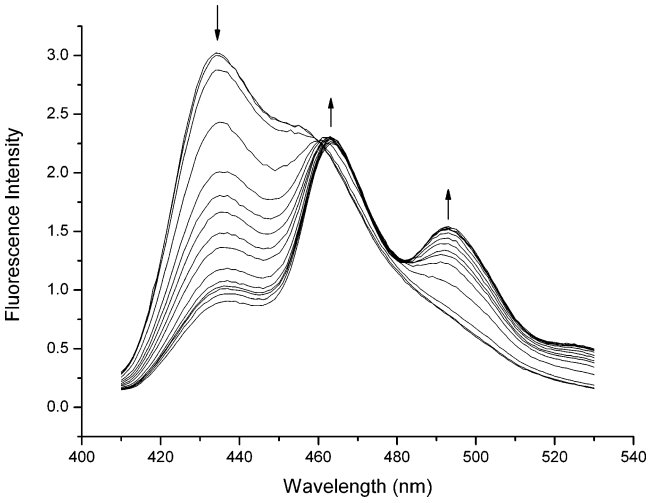

FIGURE 3. Fluorescence spectral changes of **3** with increasing chloride ion concentration.

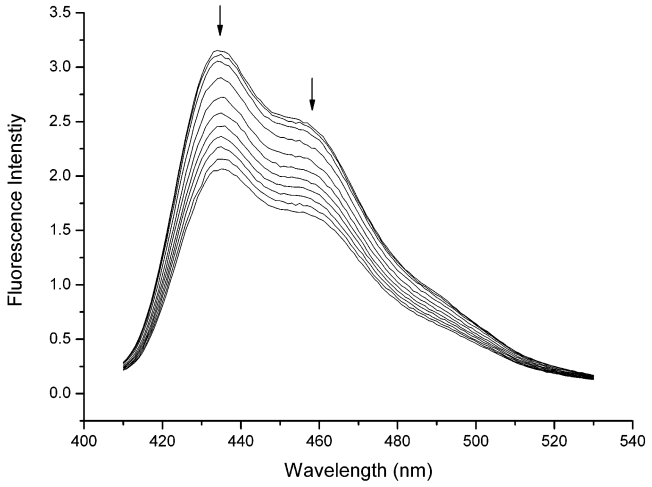

FIGURE 4. Fluorescence spectral changes of **3** with increasing bromide ion concentration.

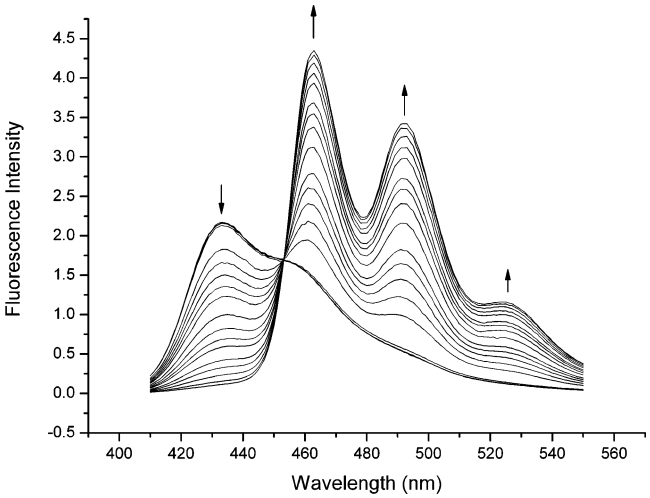

FIGURE 5. Fluorescence spectral changes of **3** with increasing benzoate ion concentration.

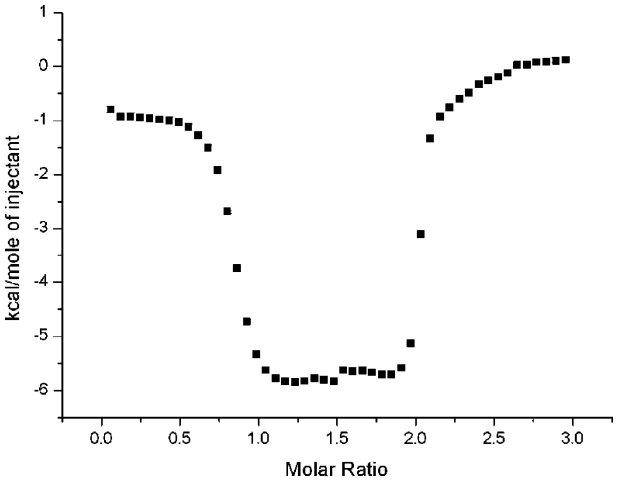

FIGURE 6. Calorimetric titration of **3** with tetrabutylammonium fluoride.

individual equivalents of chloride could not be evaluated. However, fitting of the decrease in emission at 435 nm with increasing chloride ion again fit a 1:1 stoichiometry model and gave an equilibrium constant of about 18 (±3) M^{-1}. This is reasonably consistent with the result from NMR titration. The data does not show a clear isosbestic point suggesting the presence of more than two species. This might be attributed to a small amount of deprotonation of **3** by Cl^- in addition to the association reaction and may contribute to the lack of complete agreement between association constants determined by NMR and fluorescence. Bromide caused a decrease in the fluorescence of **3** but did not induce new emission bands, consistent with the known ability of bromide ion to cause fluorescence quenching upon association with a fluorophore but indicating no significant deprotonation of **3** by bromide (Figure 4).[27] A plot of the decrease in emission at 435 nm versus [Br^-] again fit 1:1 binding and gave an association constant of 3.0 (±0.5) M^{-1}, in good agreement with the NMR result. Reaction of **3** with benzoate ion was also studied by fluorescence (Figure 5). As with fluoride, the spectrum did not change significantly until addition of the second equivalent of benzoate, and the spectral changes were essentially identical to those observed with fluoride. This indicates that **3** is also deprotonated by 2 equiv of benzoate. Similar observations have been made with other anion receptors and are attributed to formation of a hydrogen-bonded carboxylic acid−carboxylate complex.[6,8]

The reactions of **3** with fluoride and benzoate ions were further studied using isothermal titration calorimetry. With fluoride, initially the enthalpy change was about 1.0 kcal/mol (Figure 6). However, as the titration approached 1 equiv of fluoride ion, the enthalpy change per injection increased and reached a new plateau of about 5.7 kcal/mol. This enthalpy change continued until it dropped off after 2 equiv of fluoride was reached. These data further confirm the reaction with 2 equiv of fluoride. As ΔH is much greater for reaction with the second equivalent of fluoride, the initial binding step must be more entropically favorable than the deprotonation step under these conditions. The titration with benzoate also showed distinct reactions with the first and second equivalents (Figure 7). In this case, the initial 1:1 complex formation was endothermic by about 0.3 kcal/mol. The addition of the second equivalent was exothermic by about 0.16 kcal/mol, though this was essentially indistinguishable from the heat of dilution of benzoate

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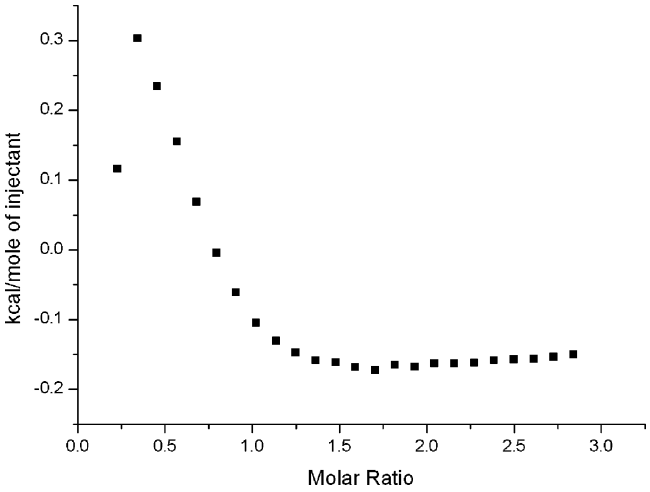

FIGURE 7. Calorimetric titration of **3** with tetrabutylammonium benzoate.

and thus no significant change in heat evolution was observed after reaching 2 equiv. For both the fluoride and benzoate titrations, curve fitting to obtain individual equilibrium constants using the data analysis software of the ITC instrument failed.

The entropy-driven association of anions to guanidinium-based receptors has been observed and is attributed to the entropically favorable desolvation of the receptor.[28−31] Reported thermodynamic analysis of stepwise reaction with 2 equiv of anion are very limited and distinct from the examples reported here. A cholic acid derivative bearing two appended urea groups gave a titration curve showing distinct phases for the first and second equivalent of fluoride, but the heat release was smaller during addition of the second equivalent.[32] The reaction may involve binding of fluoride to each urea and does not necessarily involve deprotonation. ITC analysis of dihydrogen phosphate anion addition to a bicyclic guanidinium-based receptor showed an exothermic reaction with the first equivalent of phosphate followed by an endothermic reaction with a second equivalent.[33] Our observation of reaction with the second equivalent of anion being more highly exothermic appears unique, though perhaps not surprising given the acid−base reaction occurring in the second step.

Another observation is that although the equilibrium constant for the deprotonation step in reactions of anions with their "receptors" has been expressed in M^{-1} units, this is actually a two-reactant two-product equilibrium if the receptor anion and XHX^- (or HX if deprotonated by the initial equivalent of X^-) fully dissociate. The reported (apparent) equilibrium constants presumably reflect the reciprocal of the free halide ion concentration at which the receptor complex is 50% converted to the deprotonated form ($[RHX^-] = [R^-]$). Thus, at this concentration, $[XHX^-] = [R^-] = 0.5[R]_{total}$ and the true equilibrium constant is expressed as

$$K = [R^-][XHX^-]/([RHX^-][X^-]) = 0.5K_{app}[R]_{total} \quad (1)$$

The analysis is similar for deprotonation by a single equivalent of X^-. Thus comparisons of apparent equilibrium constants for the deprotonation step are only meaningful if they are conducted at the same receptor concentration. Based on 50% conversion of the **3**−F^- complex to the deprotonated form of **3** at 10^{-6} M F^-, the true equilibrium constant is $0.5[\mathbf{3}]_{total}/10^{-6}$ M, which equals about 10 (8.75) at the total concentration of 17.5 μM **3**. The extent of deprotonation is thus governed by the anion to receptor ratio rather than just the anion concentration. In sensor applications, it should thus be possible to fine-tune sensitivity by choice of sensor concentration. Furthermore, it may be possible to limit deprotonation and thus stay within the realm of supramolecular chemistry by using a higher concentration, in addition to a less acidic receptor.

Conclusion

This work provides some new insights into the thermodynamics of anion receptor interactions in systems involving deprotonation of the receptor by the anion, as well as dependence on receptor concentration. This may clarify some issues that should be considered in the further development of anion receptors and sensors.

Experimental Section

4,5-Diacetamidoacridine-9(10*H*)-one 3. This compound was prepared as described previously.[23] ^{1}H and ^{13}C NMR spectra of **3** were in agreement with the literature except that the N−H protons were observed as two singlets at δ 9.91 (1H) and 10.25 (2H) instead of a single signal at δ 9.98 as reported.[23]

^{1}H NMR Experiments. NMR titration experiments were conducted using a 0.025 M solution of **3** in DMSO-d_6 (1.0 mL). To this solution was added a 0.025 M solution of **3** containing tetrabutylammonium chloride (1.0 M) in increments of 25, 25, 50, 50, 80, and 100 μL. For bromide titrations, a solution of **3** containing tetrabutylammonium bromide (2.0 M) was added in the same volume increments. Changes in chemical shift of the amide protons were fit to halide concentration using the program EQNMR to obtain binding constants.

Fluorescence Titrations. Fluorescence spectra were recorded at 25 °C with excitation at 360 nm. Equilibrium constants were calculated by plotting fluorescence intensity at 434 nm versus anion concentration in Origin 7.0.

For fluoride titrations, to a solution of **3** in DMSO (2.5 mL, 17.5 μM) was added a DMSO solution of tetrabutylammonium fluoride (8.0 mM) in increments of 14 × 1 μL, 3 × 2 μL, and 2 × 4 μL. For chloride titrations, to a solution of **3** in DMSO (2.5 mL, 1.75 μM) was added a DMSO solution of tetrabutylammonium chloride (1.23 M) in increments of 2 × 10 μL, 5 × 20 μL, 6 × 40 μL, and 55 μL. For bromide titrations, to a solution of **3** in DMSO (2.5 mL, 1.75 μM) was added a DMSO solution of tetrabutylammonium bromide (1.16 M) in increments of 10 μL, 20 μL, 30 μL, and 7 × 40 μL. For benzoate titrations, to a solution of **3** in DMSO (2.5 mL, 17.5 μM) was added a DMSO solution of tetrabutylammonium benzoate (2.58 mM) in increments of 6 × 4 μL, 4 × 2 μL, 4 × 4 μL followed by a 25.8 mM tetrabutylammonium benzoate solution in increments of 2 × 1 μL, 2 μL, 4 μL, and 2 × 6 μL.

Calorimetry Experiments. All calorimetry experiments were performed at 25 °C. For fluoride titration, the solution of **3** (0.87

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mM) in DMSO (1.3 mL) was titrated with 48 increments of 5 μL of tetrabutylammonium fluoride (14.0 mM) with a 200 s delay between injections. For benzoate titration, the solution of **3** (1.75 mM) in DMSO (1.3 mL) was titrated with 24 increments of 10 μL of tetrabutylammonium benzoate (25.8 mM) with a 300 s delay between injections. The initial injection gave a large exothermic peak that was omitted from the titration figure.

Acknowledgment. This work was supported by the National Science Foundation (CHE0213457). NMR facilities were supported by a grant from the National Science Foundation (CHE0131146). We thank M. J. Hynes for the EQNMR program.

JO062467J