Anthracene-Based Amido–Amine Cage Receptor for Anion Recognition under Neutral Aqueous Conditions

Boris S. Morozov,[a] Siva S. R. Namashivaya,[a] Marina A. Zakharko,[c]
Aleksandr S. Oshchepkov,[a, b] and Evgeny A. Kataev*[a, d]

Dedicated to Jean-Marie Lehn for his 80th birthday and great inspiration to develop the supramolecular chemistry

A new amido–amine cage receptor, which combines 1,8-anthracene diacarboxamide subunit and a polyammonium azamacrocyle, is reported. Bearing both the hydrogen bond donor and the acceptor binding sites, the receptor is able to bind phosphate selectively under neutral (pH 7.2) aqueous conditions. The recognition events for phosphate and dicarboxylates are accomplished by a fluorescence enhancement in the anthracene emission. As revealed by experimental and theoretical studies, phosphate and oxalate show different recognition modes. Phosphate demonstrates hydrogen bond acceptor properties, while the coordination of oxalate favours the protonation of the receptor.

Recognition and sensing of anions in an aqueous solution is an important challenge in supramolecular chemistry.[1] The fact that anions play important roles in living systems and in the environment inspires researchers to design highly selective synthetic receptors and probes. Considerable progress has been made in the area of anion recognition during the last decade.[2]

However, receptors that selectively bind and detect anions in a buffered aqueous solution remain rare.[3–9] One of the most strongly binding receptors for anions in water are azacryptands pioneered by Simmons, Park and Lehn.[4] This class of synthetic hosts has grown in a broad variety of rigid and flexible, high affinity binding receptors during the last two decades.[2b,3]

In a search of a strategy to improve the selectivity of synthetic hosts for certain anions, various modifications in their structures have been explored including the introduction of rigid fragments, methylation[6] and addition of straps into the receptor structure.[7]

Combining hydrogen bond donor and acceptor groups has been proven to be a beneficial strategy to generate high selectivity for certain series of anions.[8] This strategy could be especially advantageous for achieving a selectivity for anions carrying protons in an aqueous solution such as phosphates, carboxylates and complex organic anions.[9] Phosphate is present in water under neutral conditions as a mixture of anions H₂PO₄⁻ and HPO₄²⁻. Therefore, it is important to have both hydrogen bond acceptor and hydrogen bond donor binding sites in the structure of a host.[10] Recent examples of such type of receptors for phosphates working in water include a macrocyclic amido–amine receptors[11] imine cages[12] and azacryptands bearing pyridine or pyrrole rings.[13] Analysis of the literature shows that amide group can function as both donor and acceptor of hydrogen bonds. In this regard, 1,8-anthracene dicarboxamide fragment can be a promising building block for anion recognition. Moreover, the anthracene dye can provide fluorescence properties for a synthetic receptor. There have been only a few reports published on the use of derivatives of 1,8-anthracene dicarboxylic acids in synthetic receptors, such as recognition of dicarboxylic acids,[14] and cations.[15]

Herein, we report the design and synthesis of a new amido–amine cage receptor, which combines in its structure 1,8-anthracene diacarboxamide subunit and a polyammonium azamacrocyle. The anthracene part serves not only as a source of hydrogen bond donor NH-groups and acceptor CO-groups, but also as a fluorescent dye for anion detection. The receptor shows a good binding selectivity towards phosphate over other mono- and di-negative anions in a buffered aqueous solution at pH 7.2 (50 mM TRIS buffer). Phosphate and oxalate demonstrate the strongest fluorescence enhancement among other anions studied.
The receptor was synthesized starting from the mono-Cbz-protected tris(2-aminoethyl)amine 2 according to Scheme 1. The free NH-groups in 3 must be protected with an orthogonal Boc-group to achieve high yield at the last step – acylation with 1,8-anthracene dicarboxylic acid chloride. The attempts to acylate the derivative of polyaniline 5 lacking the protecting groups were unsuccessful. In this case, the acid chloride reacts with all free amine groups leading to a complex mixture of products. The yield of macrocyclization (20%) agrees with those reported by us previously.\(^{[11b]}\)

Despite the presence of three aromatic rings, the receptor has moderate solubility in water under neutral and acidic conditions. This fact allowed us to conduct potentiometric titrations. The stepwise \(pK_a\) values determined in 0.1 M NaCl aqueous solution are 11.0; 9.8; 7.6; 6.4; 3.3. The measurements revealed that 1 is found in 3-fold (64%) and 4-fold (24%) protonated states under neutral conditions (pH 7.2). This distribution of forms exactly fits the requirements for the fold (24 %) protonated states under neutral conditions (pH 7.2).

Fluorescence titrations were carried out with a series of inorganic acid salts and oxalate, as the anion, which induced the strongest fluorescence enhancement. The anions, such as fluoride, chloride, and nitrate did not induce any changes in fluorescence and UV-Vis titration experiments (Figure 1). Bromide and iodide induced small changes due to the dynamic quenching ability. On the contrary, di-negatives oxyanions, such as phosphate, and oxalate resulted in an enhancement of fluorescence. This effect is in a good agreement with the expected mechanism for PET anion probes. Apparent binding constants were obtained by fitting the binding isotherms with a HypSpec program (Table 1).\(^{[17]}\) The stoichiometry of binding was determined from the best fit including residual plots and separate Job Plot experiments.\(^{[18]}\) A detailed analysis of the titrations revealed that phosphate is bound with the strongest affinity (4460 M\(^{-1}\)) among other studied anions (Table 1). However, as can be inferred from Figure 2, the receptor binds phosphate in a 1:2 host-anion stoichiometry. The first binding event is accomplished with fluorescence quenching, while the coordination of the second phosphate results in a fluorescence enhancement. Apparent binding constants for phosphate obtained from the UV-Vis titrations under the same conditions were in a good agreement with those determined by fluorescence: \(\log K_{1} = 3.74 \pm 0.01\); \(\log K_{2} = 1.89 \pm 0.02\). Fitting the oxalate binding yielded \(\log K_{1} = 2.64 \pm 0.01\) (Figure 54.5).

Binding of two and more phosphates can be explained taking into account recently published crystal structures of receptors forming complexes with two and more phosphate anions.\(^{[19]}\) The multiple binding of anions is often observed with their protonated forms, which can form complexes similar to the carboxylic acid dimer. Since our receptor is highly charged,
these interactions can be relatively strong. For instance, such a 1:2 binding mode we have observed recently in the solid structure of an oxalate complex.\[20\]

Given the fact that oxalate induces the strongest emission enhancement, we tested other dicarboxylates. Interestingly, fumarate demonstrated a slightly higher binding constant as compared to that of oxalate, but similar 1.5-fold fluorescence enhancement (Figure S8). Similarity in size and geometry of these anions can explain their strong affinity and the turn-on fluorescence answer.

To reveal the coordination mode of anions with receptor 1 we have optimized the structure of complexes $\text{1H}_4^+ \cdot \text{HPO}_4^{2-}$ and $\text{1H}_4^+ \cdot \text{C}_4\text{O}_4^{2-}$ with the help of DFT calculations (PBE/ TZB).\[21\] Initially, the geometry of the tetra-protonated receptor was calculated. In order to optimize the geometry of complexes with anions, we added $\text{HPO}_4^{2-}$ and $\text{C}_4\text{O}_4^{2-}$ in the cavity with different orientations relative to the binding sites. Further analysis of the generated structures yielded two complexes with lowest energy shown in Figure 3. As expected, the oxalate anion forms hydrogen bonding interactions with protonated secondary amines, which force the anion to twist. Such a geometry was often observed experimentally in the complexes with polyammonium receptors.\[13a,23\] Oxalate forms an additional hydrogen bond with the amide group of anthracene.

The protonated state of phosphate appeared to be different from that used in the starting optimization step. As can be seen in Figure 3a, $\text{HPO}_4^{2-}$ accepted two protons from the ammonium groups and protonated the amide oxygen atom forming $\text{H}_2\text{PO}_4^-$. This acceptor property of phosphate in water was observed experimentally by different research groups\[5b,22\] and thus supports the reliability of our calculations. Notably, one amide group in involved in hydrogen bonding interactions with phosphate in a similar manner as observed in acid dimers. This additional complementary interaction might be the reason for the good selectivity for phosphate.

The results of DFT calculations helped us to understand an unusual fluorescence response for phosphate – quenching of fluorescence during the addition of the first 10 equivalents and enhancement of fluorescence with larger quantities of the anion. It is known that the more protons receptor bears, the more efficient is the blocking of the PET process.\[24\] Therefore, the fluorescence quenching can be explained by the deprotonation of the protonated secondary amines, which is induced by phosphate coordination. This fact agrees with the obtained structure of the phosphate complex. Hence, fluorescence enhancement observed in the presence of excess of phosphate could be caused by protonation via coordination of anions outside the receptor cavity.

The fluorescence enhancement observed with oxalate is in a good agreement with those results observed for the receptors reported by us previously.\[18\] This fact indicates that coordination of oxalate induces the protonation of the receptor, i.e. it increases the population of 4-fold protonation state.

Additional evidence for different coordination modes of oxalate and phosphate was obtained from $^1$H NMR titrations (50 mM TRIS buffer in D$_2$O, pH 7.2, 10% DMSO-d6). As can be inferred from Figure 4, the addition of oxalate induces strong upfield shift of the phenyl $\text{C}^\text{H^9}$ signal, while the anthracene $\text{C}^\text{H^9}$ signal was shifted downfield. The coalescence of $\text{CH}_3^\text{13}$ signals during the titration of 1 with oxalate indicates that the receptor in the complex adopts a symmetrical structure. In case of phosphate addition, we observed a strong downfield shift of the phenyl $\text{C}^\text{H^9}$ signal, while all anthracene signals were slightly shifted upfield. Overall, two anions resulted in opposite proton shifts. This interesting behavior can be rationalized by different recognition modes of phosphate and oxalate. As revealed by DFT calculations, phosphate pulls the protons from the protonated amines and this effect is reflected in a downfield
shift of an adjacent phenyl C–H*H. On the contrary, the coordination of oxalate increases the protonation degree of the receptor (i.e., shift the pK_a values of amines) and thus we observe an upfield shift in NMR titrations. Thus, the results of NMR experiments confirm the recognition mechanism suggested from fluorescence experiments and DFT calculations.

In conclusion, we have designed and synthesized an amido–amine cage receptor bearing 1,8-anthracene dicarboxamide fluorescent subunit. The receptor was found to bind amido anions from fluorescence experiments and DFT calculations. We observe an upfield shift in NMR titrations. Thus, the results of NMR experiments confirm the recognition mechanism suggested from fluorescence experiments and DFT calculations.

Acknowledgements

We thank Deutsche Forschungsgemeinschaft (DFG grant 3444/7-1 and 3444/12-1 for E.A.K.), RUDN University Program “5-100” for A.S.O. and ESF Project “Nitramon” for financial support.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: anion recognition · macrocycle · supramolecular chemistry in water · anthracene · fluorescent probe

Experimental Section

Experimental details, NMR spectra, potentiometric titrations and details to DFT calculations are given in the Supporting Information file.


Manuscript received: October 10, 2019
Revised manuscript received: November 8, 2019