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COMMUNICATION

Real-time Analysis of Multiple Anion Mixtures in Aqueous Media Using a Single Receptor

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ABSTRACT: Bambusuril-based receptors have been used in conjunction with ^{1}H NMR spectroscopy to recognize mixtures of inorganic anions in aqueous solutions. This was achieved by examining complexation-induced changes in the receptors' ^{1}H NMR fingerprints. This approach enables the simultaneous identification of up to 9 anions and the quantification of up to 5 anions using a single receptor in DMSO containing 5 % D_2O. Toxic perchlorate was recognized and quantified at 0.1 μM (1.8 ppb, mol/mol) concentration in pure water.

The design and preparation of synthetic chemosensors that respond selectively to specific analytes is of fundamental importance for monitoring biochemical processes and environmental changes.[1,2] Most of these processes take place in water which leads to development of synthetic receptors that can sense small organic molecules, metal cations, and inorganic anions in aqueous media. Despite of its recent progress, sensing of inorganic anions in water remains challenging task.[3] Inorganic anions have higher energies of solvation than cations of similar sizes and exist only at certain pH ranges.[4,5] It is therefore particularly difficult to create potent anion receptors that can overcome water-anion interactions.[6–10] Recognizing mixtures of inorganic anions in water solution is even more problematic and systems for the analysis of such mixtures based on host-guest systems are rare. There is only one recently developed solution to this problem involving the use of receptor arrays.[11–13] These arrays consist of multiple receptors, each of which responds more or less selectively to each anion. To the best of our knowledge, there is essentially no example of a single receptor molecule that would enable differentiation of multiple anion mixtures in aqueous media. Here we present such chemosensors, bambusuril derivatives **BU-1** and **BU-2** (Figure 1), single receptors that enable the detection and quantification of mixtures of ^{1}H NMR-silent anions in aqueous environment using ^{1}H NMR spectroscopy. Remarkably, this method allows sensing of iodide and perchlorate at submicromolar concentrations in pure D_2O!
Bambusurils (BU) are macrocyclic anion receptors consisting of glycoluril units connected by a set of methylene bridges (Figure 1). They are known to form stable inclusion complexes

with organic and inorganic anions.[6,14–17] The inclusion of an anion inside the BU cavity causes changes in the chemical shifts of the macrocycle's hydrogen atoms that can be followed by ^{1}H NMR spectroscopy. We have previously reported that the association of **BU-1** and halides in chloroform at room temperature is slow on the NMR time scale. The slow exchange is accompanied by the presence of separate sets of signals for the free and bound macrocycles.[17] This behavior is quite rare in interactions between anions and receptors.[18–21] Based on these findings, we hypothesized that inorganic anions with different shapes and sizes that form stable complexes with bambusuril could be differentiated based on the unique ^{1}H NMR fingerprints of the complexed macrocyles in aqueous media.
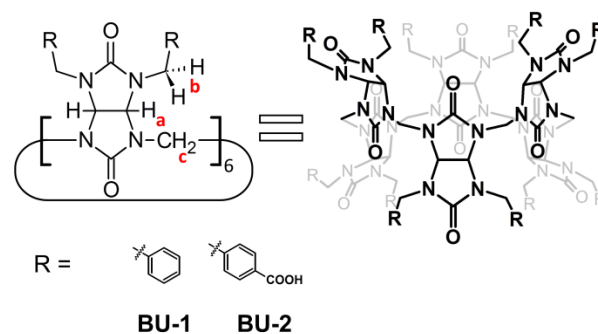

Figure 1. Structures of tested bambusuril derivatives.

To test this suggestion we investigated the interactions of 12 anions with **BU-1** in DMSO-d_6 containing 5 % D_2O. The anions investigated in this work include environmental pollutants (ClO_4^-, CN^-, ReO_4^-, NO_3^-), biologically active anions (Cl^-, HSO_4^-), and species used in ion-pairing catalysis (BF_4^-) and lithium-ion battery designs (PF_6^-). Analysis by ^{1}H NMR spectroscopy revealed that all of the selected anions induce similar behavior when added to **BU-1** solutions (Figure 2, see SI, Figure S1 for full spectra). The signals of the free and bound forms of the macrocycle were observed when less than 1 equiv. of the anion was added, demonstrating that anion exchange was slow on the NMR time scale. Moreover, when 1 equivalent of

anion was added to a submillimolar **BU-1** solution, only signals corresponding to the anion-bound form of the macrocycle were detected. The observed NMR patterns are consistent with the formation of very stable host-guest complexes of 1 : 1 stoichiometry. This is in agreement with the 1 : 1 binding mode and high affinity (> 10^5 M^{-1}) previously determined for complexes of **BU-1** with halides in chloroform.[17]
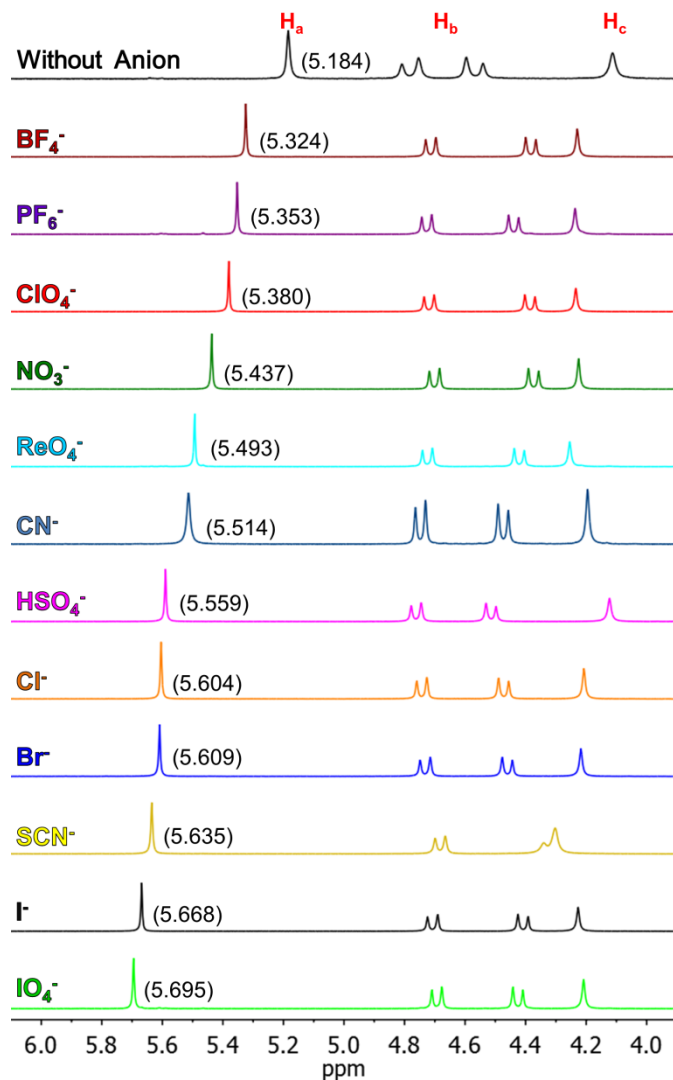

Figure 2. ^{1}H NMR spectra (500 MHz, 5% D_2O - DMSO-d_6, 30 °C, TMS) of **BU-1** ($c_{(BU-1)}$ = 1 × 10^{-3} M) in the absence and presence of 12 anions (in excess). The chemical shifts of the H_a signals in each spectrum are given in brackets. Anions were added in the form of tetrabutylammonium (TBA) salts.

Figure 2 illustrates the sensing potential of **BU-1**. First we measured the ^{1}H NMR spectrum of **BU-1** in the absence of anions. NMR spectra were then acquired for the complexes of **BU-1** with individual anions and their chemical shifts were compared. Figure 2 clearly shows that the chemical shifts of the **BU-1** protons are highly sensitive to the nature of the complexed anion. The most pronounced changes in chemical shift following anion inclusion were observed for the methine protons H_a. The difference between H_a signals for anion free macrocycle and the most downfield shifted complex is almost 0.51 ppm. If we consider only the H_a signals of the most upfield shifted versus the most downfield shifted H_a signal for

bambusuril-anion complexes (complexes with BF_4^- and IO_4^- anions) then the change drops slightly to 0.37 ppm. The macrocycle contains 12 such protons H_a, which are located on its glycoluril building blocks and project into the center of the cavity. Most of the tested anions can be distinguished based on the chemical shift of the H_a signal.

However, some pairs of anions (Br^- and Cl^-; HSO_4^- and Cl^-;) cannot be distinguished based on the chemical shifts of these methine protons alone. In the case of the HSO_4^- and Cl^- pair discrimination can be achieved by considering the unique chemical shifts of the macrocycle's H_b and H_c protons following complex formation. Spectra for each complex were acquired at different concentrations (10^{-3} - 10^{-5} M) at least three times, and the positions of the signals never changed by more than 0.005 ppm (Figure S10).

Ability of **BU-1** to sense anions in their individual aqueous solution lead us to challenge the macrocycle for qualitative analyzing multi-anion mixtures. We found out that **BU-1** is able to differentiate up to 9 anions in 5% D_2O - DMSO-d_6. Amazingly, the signals characterizing complexes of **BU-1** with anions in their mixtures were within 0.005 ppm of their location in the corresponding isolated complexes and remained constant upon dilution from 6 to 0.01 mM (Figure 3). Please note that a similar approach for analyzing multi-component anion mixtures but performed in organic solvents was reported previously by Jeong and coworkers.[21] Their indol-based receptor benefited from the changes in chemical shift of NH protons induced by hydrogen bonding interaction with anions. It is therefore unlikely this system would function in aqueous media due to proton – deuterium exchange.
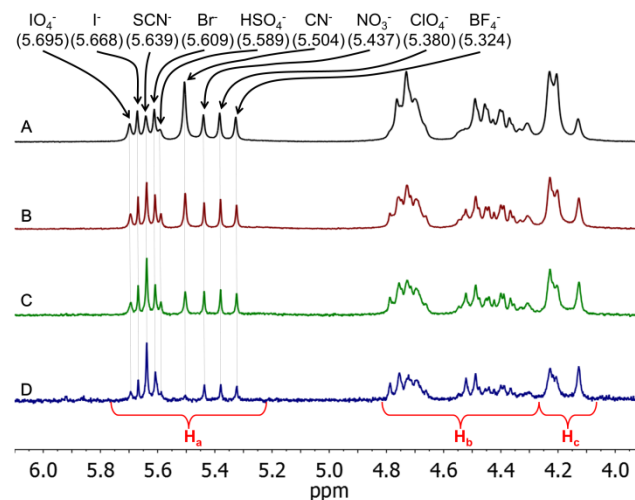

Figure 3. ^{1}H NMR spectra (500 MHz, 5% D_2O - DMSO-d_6, 30 °C, TMS) of **BU-1** - anion mixture at different concentrations: A) $c_{(BU-1)}$ = 6 × 10^{-3} M, $c_{(A)}$ = 5 × 10^{-4} M; B) $c_{(BU-1)}$ = 1.2 × 10^{-3} M, $c_{(A)}$ = 1 × 10^{-4} M; C) $c_{(BU-1)}$ = 6 × 10^{-4} M, $c_{(A)}$ = 5 × 10^{-5} M; and D) $c_{(BU-1)}$ = 1.2 × 10^{-4} M, $c_{(A)}$ = 1 × 10^{-5} M. $c_{(A)}$ is concentration of individual anions in the solution. The chemical shifts of the H_a signals in each spectrum are given in brackets.

Quantitative NMR analysis is gaining more popularity, largely due to its universal applicability for organic substrates.[22–24] Most quantitative NMR studies involve the direct observation of NMR-active nuclei within the studied molecules. This method can be used to determine the concentration and purity of agrochemicals, natural products or synthetic molecules.[25,26] In addition, there is growing interest in chromatographic NMR spectroscopy and indirect NMR sensing based on supramolecular interactions between analytes and NMR-active

receptors.[27–29] However, NMR-based approaches have not previously been used to analyze complex mixtures of small inorganic anions in aqueous media. We therefore decided to investigate the potential of **BU-1** to serve as an NMR-active quantitative chemosensor for analyzing multi-anion mixtures. We selected five anions (I^-, Br^-, NO_3^-, ClO_4^-, and BF_4^-) whose complexes with **BU-1** are readily distinguished by ^1H NMR due to the very different chemical shifts of their H_a protons (Figure 4). Mixtures of these five species with individual anion concentrations of around 10^{-4} M were added to an excess of **BU-1**.[30] Peaks occurring between 5.6 and 5.2 ppm in the mixtures' NMR spectra were confidently assigned to the complexes of BU with specific anions because their chemical shifts matched those observed on mixing **BU-1** with solutions of single anions (within experimental error). The methine signals corresponding to individual anion complexes were well separated, enabling quantification of the anions. The concentrations of each anion could therefore be determined with an experimental error of less than 10 %. The table shows the agreement between the theoretical ($c_{teor.}$) and measured ($c_{calc.}$) anion concentrations. Dimethyl sulfone was used as an internal standard for the quantitative measurements. Anions were used in the form of their tetrabutylammonium salts. Qualitative and quantitative analyses with similar levels of certainty could also be performed using mixtures of the same anions in which the anion concentration ranged from 10^{-3} - 10^{-4} M (Figure S3 and S4).

During the middle stages of this study, the bambusuril derivative **BU-2** was prepared in our group.[6] This compound is soluble in neutral and basic water at millimolar concentrations, which allowed us to evaluate the sensing ability of bambusurils in pure water.
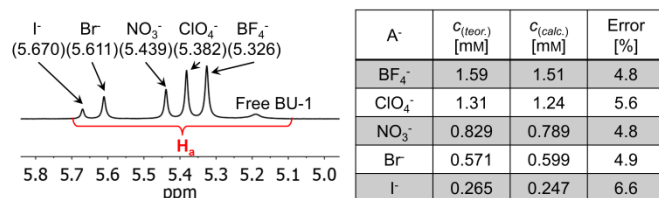

Figure 4. ^1H NMR spectra (500 MHz, 5% D_2O - DMSO-d_6, 30°C, TMS,) of **BU-1** in the presence of 5 anions showing their quantitative analysis. The inserted table shows the agreement between the theoretical ($c_{teor.}$) and measured ($c_{calc.}$) anion concentrations. Dimethyl sulfone was used as an internal standard for the quantitative measurements. Anions were used in the form of their TBA salts.

A^-	$c_{(teor.)}$ [mM]	$c_{(calc.)}$ [mM]	Error [%]
BF_4^-	1.59	1.51	4.8
ClO_4^-	1.31	1.24	5.6
NO_3^-	0.829	0.789	4.8
Br^-	0.571	0.599	4.9
I^-	0.265	0.247	6.6

We performed ^1H NMR experiments in D_2O containing 20 mM K_2DPO_4. pD of the solution was 7.1 when 1 mM concentration of **BU-2** was used. In contrast with DMSO-d_6 containing 5 % D_2O examined in this work, the binding of **BU-2** to some anions appeared to be fast on the ^1H NMR time scale in D_2O at 30°C. These anions, including F^-, Cl^-, CN^-, IO_4^-, ReO_4^-, show lower affinity to **BU-2** ($K_a < 3.0 \times 10^4$ M^{-1}). On the other hand, the remaining anions (Br^-, NO_3^-, PF_6^-, BF_4^-, I^-, ClO_4^-) form stronger complexes with the macrocycle in slow regime on the NMR time scale. Four of these strongly binding anions can be recognized upon inclusion in **BU-2** by the unique position of the H_a signal (Figure 5A-E).

Nitrate was excluded from this experiment as the induced shift of the **BU-2** H_a signals upon complexation is similar to the one of PF_6^-. Unique NMR fingerprints of **BU-2** complexes with four anions enable qualitative analysis of their mixture (Figure 5F). This is possible due to constant chemical shifts of individual complexes which remained the same also in the anion mixture.

Please note, that the recognition of multi-anion mixture were possible even at anion concentration of 10^{-5} M in D_2O. The height to width ratio of the H_a signals of anions in their mixtures can be further improved by decreasing working temperature from 30 to 5°C (Figure S7 and S8). This is obvious particularly in the case of Br^- in which case the H_a signal is not visible at 30°C but appears in the spectra recorded at 5°C.
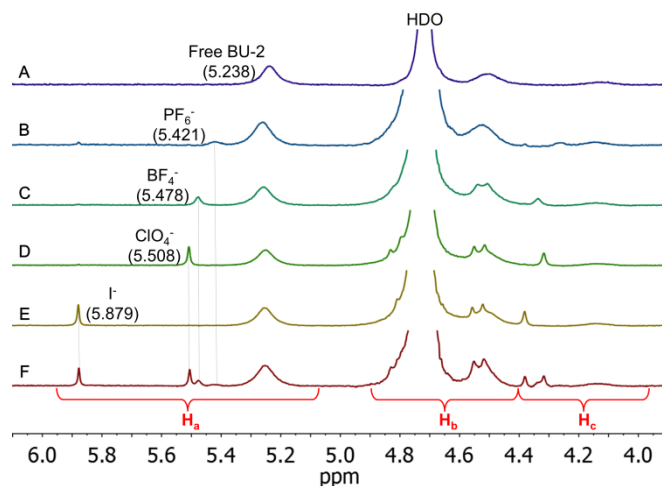

Figure 5. ^1H NMR spectra (500 MHz, D_2O, 20 mM K_2DPO_4, 30°C) of **BU-2**: A) in absence of anion, B –E) in the presence of specific anion, and F) in the presence of 4 anions. Concentrations of anions ($c_{(A)}$= 1.0×10^{-5} M) and macrocycle ($c_{(BU-2)}$= 1.0×10^{-3} M) were kept constant for all experiments. The chemical shifts of the H_a signals in each spectrum are given in brackets.

On the other hand, H_a signals for the **BU-2** complexes with I^- and ClO_4^- remain sharp even at 30°C and are well separated. Therefore, we investigated the quantitative sensing of I^- and ClO_4^- mixture in D_2O at 30°C (Figure 6).
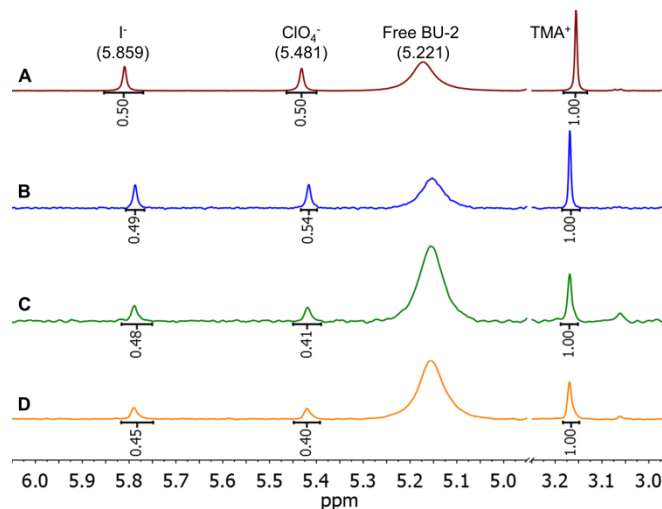

Figure 6. ^1H NMR (600 MHz, D_2O, 20 mM K_2DPO_4, 30°C) spectra of BU-2 in the presence of equimolar mixture of iodide and perchlorate with decreasing anion concentrations ($c_{(A)}$): A) $c_{(A)}$ = 8×10^{-6} M, $c_{(BU-2)}$ = 8×10^{-5} M, ns = 128, B) $c_{(A)}$ = 8×10^{-7} M, $c_{(BU-2)}$ = 8×10^{-6} M, ns = 128, C) $c_{(A)}$ = 1×10^{-7} M, $c_{(BU-2)}$ = 1×10^{-5} M, ns = 128 D) $c_{(A)}$ = 1×10^{-7} M, $c_{(BU-2)}$ = 1×10^{-5} M, ns = 1024.

We started with 8 μM concentrations of both anions in the form of tetramethylammonium (TMA) salts in the presence of 80 μM **BU-2** solution in D_2O (Figure 6A). The anion concentrations calculated from integrated area of the H_a signals agreed with the theoretical values within experimental error. Remarkably, the

detection of I^- and ClO_4^- was possible even at the individual anion concentration as low as 0.1 μM (Figure 6C and 6D).[31] Detection of such low concentrations of inorganic anions using NMR technique is unprecedented not only for aqueous but also for any organic media.

Conclusions

In conclusion, we have established a simple and straightforward approach for characterizing and quantifying mixtures of inorganic anions. This method relies on a single bambusuril receptor, **BU-1** or **BU-2**, that forms stable 1:1 supramolecular complexes with 12 different anions by enclosing them in its central cavity. The anions' shapes and sizes influence the electron density in the vicinity of the macrocycle's hydrogen nuclei, giving each complex a unique ^{1}H NMR fingerprint. The sensing system described herein is the first that enables the characterization of complex mixtures of inorganic anions by NMR in aqueous media. Importantly, it relies entirely on standard ^{1}H NMR techniques and is capable of anion sensing at submicromolar concentrations even in pure D_2O.

Acknowledgment

Notes and references

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Electronic Supplementary Information (ESI) available: Contains details of the quantitative and qualitative ^{1}H NMR analyses together with full versions of all ^{1}H NMR spectra presented in the paper. See DOI: 10.1039/c000000x/

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30   Using competitive ^{1}H NMR study and isothermal titration calorimetry we found out that **BU-1** binds BF_4^- with the lowest affinity from five tested anions with association constant of 2.2×10^5 M^{-1} (see SI). Saturated solutions of **BU-1** were used for quantitative NMR analysis. High affinity of **BU-1** together with excess of the macrocycle guarantees a quantitative anion binding during the analysis.
31   The association constant of **BU-2** with I^- and ClO_4^- under the experimental conditions are 1.0×10^7 M^{-1} and 5.5×10^7 M^{-1} respectively.[6] This means that anion binding is almost quantitative (99.0 and 99.8 %) even at 0.1 μM anion and 10 μM **BU-2** concentrations.