Real-time analysis of multiple anion mixtures in aqueous media using a single receptor†

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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-$d_6$ containing 5% D$_2$O. Toxic perchlorate was recognized and quantified at 0.1 µM (1.8 ppb, mol mol$^{-1}$) 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 its recent progress, sensing of inorganic anions in water remains a challenging task.$^3$ Inorganic anions have higher energies of solvation than cations of similar sizes and exist only in 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 (Fig. 1), single receptors that enable the detection and quantification of mixtures of $^1$H NMR-silent anions in an aqueous environment using $^1$H NMR spectroscopy. Remarkably, this method allows sensing of iodide and perchlorate at submicromolar concentrations in pure D$_2$O.

Bambusurils (BU) are macrocyclic anion receptors consisting of glycoluril units connected by a set of methylene bridges (Fig. 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 macrocycles in aqueous media.

To test this suggestion, we investigated the interactions of 12 anions with BU-1 in DMSO-$d_6$ containing 5% D$_2$O. The anions investigated in this work include environmental pollutants (ClO$_4^-$, CN$^-$, ReO$_4^-$, and NO$_3^-$), biologically active anions (Cl$^-$ and 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

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Fig. 1 Structures of tested bambusuril derivatives.
to BU-1 solutions (Fig. 2, see ESI† Fig. 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 the 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 (>10^6 M^-1) previously determined for complexes of BU-1 with halides in chloroform.17

Fig. 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. Fig. 2 clearly shows that the chemical shifts of the BU-1 protons are highly sensitive to the nature of the complexed anions. The most pronounced changes in chemical shifts following anion inclusion were observed for the Ha methine protons. The difference between Ha signals for the anion-free macrocycle and the most downfield shifted complex is almost 0.51 ppm. If we consider only the most upfield shifted Ha signals versus the most downfield shifted Hg 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 Ha protons, 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 Hg signal. However, some pairs of the 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 Hg and Hc protons following complex formation. Spectra of 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 (Fig. S10, ESI†).

Fig. 2 \(^1\)H NMR spectra (500 MHz, 5% D\(_2\)O–DMSO-d\(_6\), 30 °C, TMS) of BU-1 (c\(_{BU-1}\) = 1 x 10\(^{-3}\) M) in the absence and presence of 12 anions (in excess). The chemical shifts of the Ha signals in each spectrum are given in brackets. Anions were added in the form of tetrabutylammonium (TBA) salts.

Table 1

| Anion          | Chemical Shift (ppm) |
|----------------|----------------------|
| BF\(_4^-\)     | (5.324)              |
| PF\(_6^-\)     | (5.353)              |
| ClO\(_4^-\)    | (5.380)              |
| NO\(_3^-\)     | (5.437)              |
| ReO\(_4^-\)    | (5.493)              |
| CN\(^-\)       | (5.514)              |
| HSO\(_4^-\)    | (5.559)              |
| Cl\(^-\)       | (5.604)              |
| Br\(^-\)       | (5.609)              |
| SCN\(^-\)      | (5.635)              |
| I\(^-\)        | (5.668)              |
| IO\(_4^-\)     | (5.695)              |

Table 1 shows the chemical shifts of the Ha signals for the anion-free macrocycle and the most downfield shifted complex for each anion. 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 Hg and Hc protons following complex formation. Spectra of 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 (Fig. S10, ESI†).

Fig. 3 \(^1\)H NMR spectra (500 MHz, 5% D\(_2\)O–DMSO-d\(_6\), 30 °C, TMS) of BU-1–anion mixtures at different concentrations: (A) c\(_{BU-1}\) = 6 x 10\(^{-3}\) M, c\(_{AI}\) = 5 x 10\(^{-4}\) M; (B) c\(_{BU-1}\) = 1.2 x 10\(^{-3}\) M, c\(_{AI}\) = 1 x 10\(^{-4}\) M; (C) c\(_{BU-1}\) = 6 x 10\(^{-4}\) M, c\(_{AI}\) = 5 x 10\(^{-5}\) M; and (D) c\(_{BU-1}\) = 1.2 x 10\(^{-4}\) M, c\(_{AI}\) = 1 x 10\(^{-5}\) M. c\(_{AI}\) is the concentration of individual anions in the solution. The chemical shifts of the Ha signals in each spectrum are given in brackets.
Quantitative NMR analysis is gaining more popularity, largely due to its universal applicability for organic substrates. 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. In addition, there is growing interest in chromatographic NMR spectroscopy and indirect NMR sensing based on supramolecular interactions between analytes and NMR-active receptors. 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 (F-, Br-, NO3-, ClO4-, and BF4-) whose complexes with BU-1 are readily distinguished by 1H NMR due to the very different chemical shifts of their Hα protons (Fig. 4). Mixtures of these five species with individual anion concentrations of around 10^(-4) M were added to an excess of BU-1. 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 the experimental error). The methane signals corresponding to the 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_{theo}) and measured (c_{calc}) anion concentrations.

During the middle stages of this study, the bambusuril derivative BU-2 was prepared by our group. 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.

We performed 1H NMR experiments in D2O containing 20 mM K2PO4, pH of the solution was 7.1 when 1 mM concentration of BU-2 was used. In contrast with DMSO-d6 containing 5% D2O examined in this work, the binding of BU-2 to some anions appeared to be fast on the 1H NMR time scale in D2O at 30 °C.

These anions, including F-, Cl-, CN-, IO4-, and ReO4-, show lower affinity for BU-2 (K_a < 3.0 x 10^4 M^-1). On the other hand, the remaining anions (Br-, NO3-, PF6-, BF4-, I-, and ClO4-) form stronger complexes with the macrocycle in a 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 Hα signal (Fig. 5A–E).

Nitrato was excluded from this experiment as the induced shift of the BU-2 Hα signals upon complexation is similar to the one of PF6-. Unique NMR fingerprints of BU-2 complexes with four anions enable qualitative analysis of their mixture (Fig. 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 mixtures was possible even at an anion concentration of 10^(-5) M in D2O. The height to width ratio of the Hα signals in their mixtures can be further improved by decreasing working temperature from 30 to 5 °C (Fig. S7 and S8, ESI†). This is obviously particular in the case of Br^- in which case the Hα signal is not visible at 30 °C but appears in the spectra recorded at 5 °C.

On the other hand, Hα signals for the BU-2 complexes with I^- and ClO4^- remain sharp even at 30 °C and are well separated. Therefore, we investigated the quantitative sensing of I^- and ClO4^- mixtures in D2O at 30 °C (Fig. 6). 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 D2O (Fig. 6A). The anion concentrations calculated from the integrated area of the Hα signals agreed with the theoretical values within the experimental error. Remarkably, the detection of I^- and ClO4^- was possible even at the individual anion concentrations as low as 0.1 μM (Fig. 6C and D). Detection of such low concentrations of inorganic anions using the NMR technique is unprecedented not only for aqueous but also for any organic media.
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 1H 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 1H NMR techniques and is capable of anion sensing at submicromolar concentrations even in pure D2O.

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30. Using the competitive 1H NMR study and isothermal titration calorimetry, we found out that BU-1 binds BF4− with the lowest affinity out of the five tested anions with an association constant of 2.2 × 10^5 M^−1 (see ESIF). 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 ClO4− under the experimental conditions are 1.0 × 10^5 M^−1 and 5.5 × 10^4 M^−1, respectively. 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.