Anion binding by biotin[6]uril in water†

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In this contribution we show that the newly discovered 6 + 6 biotin-formaldehyde macrocycle Biotin[6]uril binds a variety of anionic guest molecules in water. We discuss how and why the anions are bound based on data obtained using NMR spectroscopy, mass spectrometry, isothermal titration calorimetry (ITC), computational calculations and single crystal X-ray crystallography.

Molecular recognition in water is particularly difficult to achieve due to the competitive nature of water as a hydrogen bond donor and acceptor.1 One may argue that medicinal chemistry relies heavily on supramolecular chemistry in water, which highlights the importance of pursuing new fundamental understanding within this particular area.2 In this paper we address the challenge of recognising anions in water.3

A large number of elegant and complex receptors have been prepared and studied for their molecular recognition properties. In supramolecular chemistry the most utilised receptors are those that are easily prepared, naturally occurring or commercially available. Among these are symmetrical macrocyclic structures such as calix[n]arenes,4 calix[n]pyrroles,5 and calix[n]resorcinarenes.6 Naturally occurring receptors such as the cyclodextrins7 and linear amyloses have also been studied.8 Recently a number of urea-based macrocycles,9 such as cucurbit[n]urils,10 hemicucurbit[n]urils11 and bambus[n]-urils12 have been introduced and especially the family of cucurbit[n]urils have become popular due to the rich supramolecular chemistry offered by these structures in water.13

Common to many of the popular macrocycles is that they are prepared by simple condensation reactions using aldehydes as a condensation partner. To introduce attractive features such as chirality and enhanced water-solubility into these structures subsequent and sometimes elaborate synthetic efforts are necessary. We recently introduced a new type of receptor molecule that is easily prepared in a single synthetic step, is chiral, is water soluble and is capable of binding anions in water: the Biotin[6]uril (Fig. 1).14

In this contribution we show how Biotin[6]uril is capable of binding a series of monovalent anions at pH 7.5 in phosphate buffer with binding constants ranging from log $K$ = 1.8 (Cl$^-$) and log $K$ = 4.5 ($\text{SCN}^-$). Initially we investigated a wide range of potential guest molecules and ions for their binding properties

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† Electronic supplementary information (ESI) available: Binding studies, X-ray crystal structure and Job plots. CCDC 1029718. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4ob02211d
towards the Biotin[6]uril using $^1$H NMR spectroscopy. Binding was indicated by a change in the chemical shifts of the protons on the convex side of each biotin unit pointing into the cavity (protons b and f in Fig. 2c). We tested a series of aliphatic amines (e.g. 1,6-diaminohexane, 1,7-diaminoheptane, ethanolamine, and propargylamine), a series of cations (e.g. Na+, K+, and Cs+), a series of anions (e.g. CN$^-$, ClO$_4^-$, PF$_6^-$, PhCO$_2^-$ and CH$_3$CO$_2^-$ and the anions in Table 1) and a series of neutral guests (propionitrile, CO$_2$, CS$_2$ and propargylalcohol) for binding by Biotin[6]uril in water at pH 7.5 in 100 mM phosphate buffer.

We were pleased to find that Biotin[6]uril interacts with a range of singly charged anions (Table 1). No binding to divalent anions was observed, as exemplified by experiments with SO$_4^{2-}$, WO$_4^{2-}$, CO$_3^{2-}$ and HPO$_4^{2-}$. For the series of singly charged anions we measured the binding stoichiometries using the continuous variation method of Job by means of $^1$H NMR spectroscopy in water at pH 7.5 in 100 mM phosphate buffer (Fig. 2b). All the anions presented here showed a 1:1 binding stoichiometry. To confirm that the chemical shift changes were not due to aggregation events we measured the $^1$H NMR spectra at different concentrations (ESI†) where no changes were observed as a function of concentration. To further evaluate the binding interactions of Biotin[6]uril with the anions we proceeded to measure the binding affinities using both $^1$H NMR titrations and ITC data collected at 30 °C.

<table>
<thead>
<tr>
<th>Anion</th>
<th>$^1$H-NMR $\log(K_a)$</th>
<th>ITC $\log(K_a)$</th>
<th>$\Delta H^b$ (kJ mol$^{-1}$)</th>
<th>$\Delta S^b$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>1.8 (1.7$^a$)</td>
<td>1.5 (1.0$^b$)</td>
<td>−30.7</td>
<td>−21.8</td>
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<tr>
<td>NaBr</td>
<td>3.0</td>
<td>2.7</td>
<td>−37.5</td>
<td>−21.6</td>
</tr>
<tr>
<td>NaI</td>
<td>3.7</td>
<td>3.4</td>
<td>−42.8</td>
<td>−23.0</td>
</tr>
<tr>
<td>KI$^+$</td>
<td>3.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CsI</td>
<td>3.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>1.9</td>
<td>1.7</td>
<td>−32.3</td>
<td>−22.2</td>
</tr>
<tr>
<td>NaN$_3$</td>
<td>2.9</td>
<td>2.6</td>
<td>−31.1</td>
<td>−16.1</td>
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<tr>
<td>NaClO$_4$</td>
<td>2.7</td>
<td>2.4</td>
<td>−33.3</td>
<td>−19.5</td>
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<tr>
<td>KCNO</td>
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<td>1.8</td>
<td>−29.9</td>
<td>−19.6</td>
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<tr>
<td>KSeCN</td>
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<td>4.0</td>
<td>−37.7</td>
<td>−14.5</td>
</tr>
<tr>
<td>NaSCN</td>
<td>4.5</td>
<td>4.1</td>
<td>−35.0</td>
<td>−11.2</td>
</tr>
</tbody>
</table>

$^a$ Data was obtained at pH 10.8 in carbonate buffer. $^b$ $\Delta H$ and $\Delta S$ are from the ITC data at 30 °C. All data obtained had less than 6% error.
constants (were obtained. By applying non-linear curve fitting the binding values for the protons on the convex side of each biotin unit (Table 1 and ESI†). Thiocyanate (SCN−) binds more strongly than cyanate does. (Fig. 2c) and Isothermal Calorimetric Titration (ITC, Fig. 3). The binding affinity data are summarised in Table 1.

In our previous work we have reported two single crystal X-ray structures of Biotin[6]uril.14 In one of those structures an iodide anion was bound in the binding cavity, and in the other structure a molecule of ethanol was situated in the cavity. Herein we report a new single crystal X-ray structure of Biotin[6]uril.
In this structure the cavity contains two molecules of water that are hydrogen bonded to each. On the outside of the binding cavity we observe further molecules of water (disordered) to one side, and a carboxylic acid moiety from one of the side arms of the Biotin[6]uril to the other side. The two water molecules that reside within the cavity must be replaced by the anions upon binding of the anion to the cavity.

While the cavities of the Biotin[6]uril containing water, EtOH and iodide appear similar the cavities are actually subtly different. In Fig. 5c the urea-containing five membered rings of the each Biotin unit (including the H’s that point into the binding cavity) and the connecting formaldehyde derived CH$_2$-groups are shown (overlaid). This shows that the radius of the binding pocket is relatively unaffected by the different guest molecules. It is, however, possible for the biotin units to rotate slightly within the macrocyclic structure, thus changing slightly the directionality of the C–H bonds with respect to the centre of the cavity, and also the length of the binding cavity. The cavity size is similar to that of Bambus[6]uril reported by Sindelar and co-workers.\textsuperscript{12}

One can view the centre of Biotin[6]uril as a cylinder shaped binding cavity defined by the 12 hydrogen atoms originating from the six C–H bonds from the biotin units. The 12 H-atoms define two offset circles with 6 H-atoms at the rim of each circle. By measuring the radius of each of these circles and the distance between them we get a cylinder shaped cavity with a volume of 86–102 Å$^3$ (see ESI† for details) for the three X-ray structures. This internal volume is not constant for the three crystal structures, indicating that the binding cavity does have some flexibility. This iodide containing structure has a volume of 93 Å$^3$, the EtOH containing structure a volume of 102 Å$^3$ and the water containing cavity a volume of 86 Å$^3$. This difference in cavity sizes are mainly due to the six biotin units of the macrocycle tilting slightly, giving a longer more narrow binding pocket.

Finally we studied the Biotin[6]uril-anion complexes using electrospray ionisation mass spectrometry. Solutions of Biotin[6]uril and an excess of the various anions were prepared in water, and these were analysed by direct injection ESI mass spectrometry. The spectra convincingly indicate the formation of complexes of the Biotin[6]uril and the anions. For the halide series of anions the mass spectra are shown in Fig. 6. In all three cases (Cl$^-$, Br$^-$ and I$^-$) clear molecular ions for the 1:1 complexes are observed.
Conclusions

In this communication we show how it is possible to bind a series of simple mono-charged anions to our recently discovered anion receptor Biotin[6]uril in water. We notice that the cavity of the Biotin[6]uril contains two water molecules, which upon release could contribute favourably to the enthalpically driven binding (non-classical hydrophobic effect). The enthalpically driven binding event is evident from the ITC data. The binding of anions, we speculate, is governed by a delicate balance between the anions size in order to fit in the cavity, and the hardness/softness of the anion.

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Notes and references