Recognition in water of bioactive substrates by a sulphonato $p$-tert-butylcalix[5]arene

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The molecular recognition in water of neurotransmitter monoamine and trace amine hydrochlorides by $p$-tert-butylcalix[5]-arene 1 – bearing 4-sulphonatobutoxy groups at the lower rim – has been investigated. According to $^1$H NMR measurements, the hydrophobic cavity of receptor 1 best binds tyramine hydrochloride, $K_a = 5370 \pm 870 \text{M}^{-1}$.

Keywords: water-soluble calixarene; neurotransmitters; trace amines; molecular recognition

Introduction

The classical monoamine neurotransmitters (dopamine, serotonin, norepinephrine and histamine), the structurally related trace amines (2-phenylethylamine, tyramine and tryptamine) and their G protein-coupled receptors play a key role in the physiological nervous system cellular signalling processes and in many pathological conditions, including Parkinson’s disease and schizophrenia (1). Despite their biological relevance, little is known about the monoamine-receptor binding mechanism taking place in the synaptic cleft. A number of artificial receptors (2) have been synthesised over the past few decades with a view to gaining a better understanding of the binding pocket of G protein-coupled receptors and, as an ultimate goal, developing drugs for therapeutic purposes and/or designing sensing agents for diagnostic clinical chemistry. In fact, levels of monoamine neurotransmitters and/or their metabolites in cerebrospinal fluids, plasma and urine are commonly monitored to assess not only disorders of the nervous system, but also hypotension, hypertension and other pathologies such as neuroblastoma and pheochromocytoma (3).

For some time we have been involved in the fine-tuning of macrocyclic compounds of different types and size in an on going effort to improve and enhance their properties as molecular/ion receptors (4). Within this research frame, $p$-tert-butylcalix[5]arene derivatives have been shown to effectively bind, among others, ammonium group-containing bioactive substrates in organic media (5) but, up to now, very little has been reported on their host–guest properties in water. Recently, we published a preliminary account (6) of the aggregating behaviour of a new water-soluble calix[5]arene bearing 4-sulphonatobutoxy groups at the narrow rim (see structure 1), for which a 0.64 mM critical micellar concentration (cmc) was determined in D$_2$O by means of DOSY experiments. In the same study it was also shown that this calixarene – upon endo-cavity inclusion of an n-dodecylammonium ion – yields a supramolecular amphiphile (7) displaying a cmc value about 30-fold lower.

![Diagram]

Intrigued by this unexpected ability of calix[5]arene 1 to bind primary alkylammonium ions in a highly competitive solvent such as water (8), we inevitably focused our attention on its potential behaviour as an artificial receptor of biologically active molecules (e.g. monoamine neurotransmitters and trace amines) and we now wish to report the findings of this screening on a number of them (Figure 1).

Results and discussion

Owing to a pronounced tendency of sulphonato calixarene 1 to aggregate in water, $^1$H NMR binding studies were carried out at 298 K in D$_2$O solutions where the effective concentration of the host, as a monomeric species, had been determined beforehand so that, each time, the appropriate amount of substrate under evaluation could be
added, to reach a final 1:1 host/guest ratio. To this end, a quantification protocol (9) relying on a stem coaxial NMR tube loaded with maleic acid, as a quantitative internal reference, was used. Under the experimental conditions routinely used, the effective concentration of sulphonato calixarene 1 is present in a 0.4 mM solution in the non-aggregated form (i.e. as a monomeric species readily available to undergo 1:1 endo-cavity complexation with a given substrate) was estimated to be 0.22 mM. Results obtained upon addition of 1 equiv. of substrates 2–8 to solutions of calixarene 1 are summarised in Table 1. As in the case of p-tert-butylcalix[5]arene analogues and linear alkylammonium ions in organic solvents (5c, 5d), where non-covalent interactions (\( {\text{NH}}_3\cdots{\text{O}} \) and CH\( \pi \)) are operating in concert (10), binding of guests 2–5 inside the cavity of calixarene 1 was found to be slow on the NMR time scale (Figure 2 in the case of 3). As a result, association constants \( (K_a) \) were determined by direct integration of convenient \(^1\)H NMR signals of free and complexed species of host and guest. Guest inclusion, within the \( \pi \)-rich cavity of 1, causes significant complexation-induced shifts (CISs) to the resonances of both molecules, the most prominent and diagnostic (5b, 11) being those observed for the \( \alpha \)- and \( \beta \)-CH\( _2 \) groups of the guest (Table 1), which experience the strongest shielding from the calixarene aromatic cavity.



\[ K_a \text{ data in Table 1 indicate that calixarene 1 behaves as a powerful and selective receptor of arylethylammonium ion-containing guests (Pea-HCl, 2; Tyrm-HCl, 3 and Dopa-HCl, 4). The receptor shows a peak affinity for Tyrm-HCl (} K_a = 5370 \pm 870 \text{ M}^{-1} \text{), recognises Dopa-HCl and Pea-HCl equally well (} K_a = 2043 \pm 154 \text{ and } 2205 \pm 302 \text{ M}^{-1}, \text{ respectively) and is also able to bind Sert-HCl, although less effectively (} K_a = 1167 \pm 131 \text{). A structural comparison between Tyrm-HCl and Dopa-HCl suggests that the second hydroxyl group present on the aromatic ring of the latter may cause steric hindrance upon complexation. The second OH group, in addition, enhances the hydrophilic character of the guest and consequently, with respect to Tyrm-HCl, Dopa-HCl is more willing to remain in bulk water rather than move to a lipophilic environment such as that provided by the calixarene cavity. The thermodynamic parameters \((\Delta H^\circ \text{ and } \Delta S^\circ)\) for the formation of the inclusion complexes with Tyrm-HCl (3) and Dopa-HCl (4) were calculated from the corresponding van’t Hoff plots, derived from sets of \(^1\)H NMR measurements carried out in the 278–328 K temperature range (Figure 3). In both cases, the binding process was found to be enthalpically driven and the pertinent energy values were almost identical (\( \Delta H^\circ = -28.0 \text{ and } -29.3 \text{ kJ mol}^{-1} \text{ for 3 and 4, respectively). Both complexations, on the other hand, display an unfavourable entropic contribution (}\Delta S^\circ = -19.8 \text{ and } -34.9 \text{ J mol}^{-1} \text{ K}^{-1} \text{ for 3 and 4, respectively), probably because desolvation of the host and guest molecules cannot compensate for the loss of conformational freedom of the individual components.} \]

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**Table 1.** Association constants \( (K_a) \) determined\(^a\) by \(^1\)H NMR (500 MHz, in \( \text{D}_2\text{O}, \text{at 298 K}) \), between sulphonato calix[5]arene 1 (0.22 mM) and the different amine hydrochlorides (1 equiv.) and, where appropriate, CIS\(^b\) of the methylene groups of guests.

|              | Pea-HCl | Tyrm-HCl | Dopa-HCl | Sert-HCl | Hist-2HCl | Nore-HCl | Gaba-HCl |
|--------------|---------|----------|----------|----------|-----------|----------|----------|
| \( K_a \text{ [M}^{-1}] \) | 2205 \( \pm 302 \) | 5370 \( \pm 870 \) | 2043 \( \pm 154 \) | 1167 \( \pm 131 \) | n.o.\(^c\) | n.o.\(^c\) | n.o.\(^c\) |
| \( \Delta \delta \text{ (} \alpha\text{-CH}_2 \text{)} \text{ [ppm]} \) | 3.69 | 3.70 | 3.71 | 3.41 | n.o.\(^c\) | n.o.\(^c\) | n.o.\(^c\) |
| \( \Delta \delta \text{ (} \beta\text{-CH}_2 \text{)} \text{ [ppm]} \) | 3.64 | 3.64 | 3.64 | 3.64 | n.o.\(^c\) | n.o.\(^c\) | n.o.\(^c\) |

\(^a\)\( K_a \) values derive from an average of three independent measurements.

\(^b\)CIS values were computed as the difference between the chemical shifts of methylene group resonances belonging to the guest in the free and complexed form.

\(^c\)n.o. stands for not observed.
As mentioned earlier, calixarene 1 forms, in the presence of Pea·HCl, a stable 1:1 endo-cavity complex (Table 1). However, a close inspection of the aromatic region of the $^1$H NMR spectrum (Figure S1 in the Supplementary Material) reveals a collateral host–guest interaction, most likely of an exo-cavity nature, causing an upfield shift on the resonances of the guest ($^{12}$). The reduced affinity observed for Sert·HCl, on the other hand, is consistent with the encumbrance caused by the indole moiety, which hampers the entry of the ethylammonium moiety inside the receptor cavity and, consequently, prevents the NH$_3^+$ group from reaching its ideal docking position in the vicinity of the phenolic oxygen atoms. Compared with the other substrates, the smaller CIS value detected for the $\alpha$-CH$_2$ of Sert-HCl (Table 1), upon complexation, is in line with this hypothesis. The inability of receptor 1 to bind the hydrochloride salt of (R)-(−)-norepinephrine is most probably due to the hydroxyl group next to the ammonium head precluding – for sterical reasons (13) – complementary host–guest matching altogether. No hint of endo-cavity complex formation was observed in the case of Gaba·HCl despite its aliphatic nature, linear shape and reduced size. The same was true for the dicationic substrate Hist·2HCl. In this last case, however, a downfield shift of the imidazole ring resonances (see Figure S4 in the Supplementary Material), not compatible with an endo-cavity inclusion phenomenon, indicates an alternative type of interaction (e.g. electrostatic, via the sulphonato groups of the receptor).$^3$

Findings related to Gaba·HCl and Hist·2HCl were rather unexpected but, ultimately, most welcome as they represent an additional bonus in favour of calixarene 1 as a powerful and selective synthetic receptor of monoamine neurotransmitter/trace amines in water. In analogy to the calix[6]arene-based receptor recently described by Reinaud and co-workers (8a), our findings seem to suggest that, providing no collateral host–guest interactions take place (as in the case of Pea-HCl), substrates are best recognised and included inside the apolar cavity of $p$-tert-butylcalix[5]sulphonate 1 on the basis of their increasing lipophilic character (Tyrm-HCl > Dopa-HCl > Sert-HCl).

Conclusions

According to these preliminary data, the preorganised $\pi$-rich cavity of water-soluble calix[5]arene 1 mimics the...
hydrophobic binding pockets of the G protein-coupled receptors of 2-phenylethylamine, tyramine, dopamine and serotonin and in so doing, selectively binds the hydrochloride salt of these neurotransmitters and trace amines in aqueous media. These features make receptor 1 a promising candidate for a later development of sensing devices capable of directly monitoring the level of biogenic amines in biological fluids.

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Notes
1. Besides our earlier assessment (6), the proclivity of 1 to self-assemble has now been confirmed by dynamic light scattering measurements indicating the presence of aggregates with a hydrodynamic radius of about 100 nm, even in a 0.1 mM aqueous solution. Species of this size fall outside the detection range of 1H NMR spectroscopy.
2. D2O solutions of 1 (0.4 mM) were left to equilibrate at room temperature for 1 h and then filtered through a 0.1 μm membrane filter prior to determination of their effective concentration (see the Supplementary Material).
3. Characterisation of these species is currently in progress and pertinent details will be reported in due course.

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